

314

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
19 May 2005 (19.05.2005)

PCT

(16) International Publication Number
WO 2005/045034 A2

(51) International Patent Classification⁷: C12N 15/11,
C12P 19/34, C07H 21/02, 21/04, A01N 43/04, A61K
31/713

Berthoud, CO 80513 (US). BEIGELMAN, Leonid
[US/US]; 5530 Colt Drive, Longmont, CO 80027 (US).

(21) International Application Number:
PCT/US2004/017630

(74) Agent: TERPSTRA, Anita, J.; McDonnell Boehnen Hul-
bert and Berghoff, 300 South Wacker Drive, Suite 3100,
Chicago, IL 60606 (US).

(22) International Filing Date: 3 June 2004 (03.06.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/693,059 23 October 2003 (23.10.2003) US
10/698,311 31 October 2003 (31.10.2003) US
10/720,448 24 November 2003 (24.11.2003) US
10/727,780 3 December 2003 (03.12.2003) US
10/757,803 14 January 2004 (14.01.2004) US
60/543,480 10 February 2004 (10.02.2004) US
10/780,447 13 February 2004 (13.02.2004) US
10/826,966 16 April 2004 (16.04.2004) US
PCT/US04/13456 30 April 2004 (30.04.2004) US
PCT/US04/16390 24 May 2004 (24.05.2004) US

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG,
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM,
ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,
FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI,
SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, ML, MR, NE, SN, TD, TG).

(71) Applicant (for all designated States except US): SIRNA
THERAPEUTICS, INC. [US/US]; 2950 Wilderness
Place, Boulder, CO 80301 (US).

Published:

— without international search report and to be republished
upon receipt of that report

(72) Inventors; and

(75) Inventors/Applicants (for US only): MCSWIGGEN,
James [US/US]; 4866 Franklin Drive, Boulder, CO
80301 (US). HAEBERLI, Peter [US/US]; 705 7th Street,

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: RNA INTERFERENCE MEDIATED TREATMENT OF PARKINSON DISEASE USING SHORT INTERFERING NU-
CLEIC ACID (siNA)

(57) Abstract: The present invention concerns methods and reagents useful in modulating Parkinson genes, for example, PARK1 (SNCA), PARK2, PARK7, and/or PARK5 gene expression in a variety of applications, including use in therapeutic, diagnostic, target validation, and genomic discovery applications. Specifically, the invention relates to small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules capable of mediating RNA interference (RNAi) against SNCA gene expression and/or activity. The small nucleic acid molecules are useful in the diagnosis and treatment of Parkinson Disease (PD), and any other disease or condition that responds to modulation of PARK1 (SNCA), PARK2, PARK7, and/or PARK5 expression or activity.



WO 2005/045034 A2

**RNA INTERFERENCE MEDIATED TREATMENT OF PARKINSON DISEASE
USING SHORT INTERFERING NUCLEIC ACID (siNA)**

This application is a continuation-in-part of International Patent Application No. PCT, filed May 24, 2003, which is a continuation-in-part of U.S. Patent Application No. 10/826,966, filed April 16, 2004, which is continuation-in-part of U.S. Patent Application No. 10/757,803, filed January 14, 2004, which is a continuation-in-part of U.S. Patent Application No. 10/720,448, filed November 24, 2003, which is a continuation-in-part of U.S. Patent Application No. 10/693,059, filed October 23, 2003, which is a continuation-in-part of U.S. Patent Application No. 10/444,853, filed May 23, 2003, which is a continuation-in-part of International Patent Application No. PCT/US03/05346, filed February 20, 2003, and a continuation-in-part of International Patent Application No. PCT/US03/05028, filed February 20, 2003, both of which claim the benefit of U.S. Provisional Application No. 60/358,580 filed February 20, 2002, U.S. Provisional Application No. 60/363,124 filed March 11, 2002, U.S. Provisional Application No. 60/386,782 filed June 6, 2002, U.S. Provisional Application No. 60/406,784 filed August 29, 2002, U.S. Provisional Application No. 60/408,378 filed September 5, 2002, U.S. Provisional Application No. 60/409,293 filed September 9, 2002, and U.S. Provisional Application No. 60/440,129 filed January 15, 2003. This application is also a continuation-in-part of US Patent Application No. 10/698311, filed October 31, 2003. This application is also a continuation-in-part of International Patent Application No. PCT/US04/13456, filed April 30, 2004, which is a continuation of Patent Application No. 10/780,447, filed February 13, 2004, which is a continuation-in-part of US Patent Application No. 10/427,160, filed April 30, 2003, which is a continuation-in-part of International Patent Application No. PCT/US02/15876 filed May 17, 2002, which claims the benefit of U.S. Provisional Application No. 60/362,016, filed March 6, 2002, and U.S. Provisional Application No. 60/292,217, filed May 18, 2001. This application is also a continuation-in-part of U.S. Patent Application No. 10/727,780 filed December 3, 2003. This application also claims the benefit of U.S. Provisional Application No. 60/543,480 filed February 10, 2004. The instant application claims the benefit of all the listed applications, which are hereby incorporated by reference herein in their entireties, including the drawings.

Field Of The Invention

The present invention concerns compounds, compositions, and methods for the study, diagnosis, and treatment of traits, conditions and diseases that respond to the modulation of Parkinson genes, for example, PARK1 (SNCA), PARK2, PARK7, and/or
5 PARK5 gene expression and/or activity. The present invention is also directed to compounds, compositions, and methods relating to traits, diseases and conditions that respond to the modulation of expression and/or activity of genes involved in Parkinson disease gene expression pathways or other cellular processes that mediate the maintenance or development of such traits, diseases and conditions. Specifically, the
10 invention relates to small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules capable of mediating RNA interference (RNAi) against Parkinson genes, e.g., PARK1 (SNCA), PARK2, PARK7, and/or PARK5 gene expression. Such small nucleic acid molecules are useful, for
15 example, in providing compositions for treatment or prevention of traits, diseases and conditions that can respond to modulation of Parkinson gene expression in a subject, such as Parkinson disease.

Background Of The Invention

The following is a discussion of relevant art pertaining to RNAi. The discussion is
20 provided only for understanding of the invention that follows. The summary is not an admission that any of the work described below is prior art to the claimed invention.

RNA interference refers to the process of sequence-specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs) (Zamore *et al.*, 2000, *Cell*, 101, 25-33; Fire *et al.*, 1998, *Nature*, 391, 806; Hamilton *et al.*, 1999, *Science*, 286, 950-951; Lin *et al.*, 1999, *Nature*, 402, 128-129; Sharp, 1999, *Genes &*
25 *Dev.*, 13:139-141; and Strauss, 1999, *Science*, 286, 886). The corresponding process in plants (Heifetz *et al.*, International PCT Publication No. WO 99/61631) is commonly referred to as post-transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of post-transcriptional gene silencing is thought to be
30 an evolutionarily-conserved cellular defense mechanism used to prevent the expression

of foreign genes and is commonly shared by diverse flora and phyla (Fire *et al.*, 1999, *Trends Genet.*, 15, 358). Such protection from foreign gene expression may have evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or from the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA. The presence of dsRNA in cells triggers the RNAi response through a mechanism that has yet to be fully characterized. This mechanism appears to be different from other known mechanisms involving double stranded RNA-specific ribonucleases, such as the interferon response that results from dsRNA-mediated activation of protein kinase PKR and 2',5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L (see for example US Patent Nos. 6,107,094; 5,898,031; Clemens *et al.*, 1997, *J. Interferon & Cytokine Res.*, 17, 503-524; Adah *et al.*, 2001, *Curr. Med. Chem.*, 8, 1189).

The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as dicer (Bass, 2000, *Cell*, 101, 235; Zamore *et al.*, 2000, *Cell*, 101, 25-33; Hammond *et al.*, 2000, *Nature*, 404, 293). Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs) (Zamore *et al.*, 2000, *Cell*, 101, 25-33; Bass, 2000, *Cell*, 101, 235; Bernstein *et al.*, 2001, *Nature*, 409, 363). Short interfering RNAs derived from dicer activity are typically about 21 to about 23 nucleotides in length and comprise about 19 base pair duplexes (Zamore *et al.*, 2000, *Cell*, 101, 25-33; Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188). Dicer has also been implicated in the excision of 21- and 22-nucleotide small temporal RNAs (stRNAs) from precursor RNA of conserved structure that are implicated in translational control (Hutvagner *et al.*, 2001, *Science*, 293, 834). The RNAi response also features an endonuclease complex, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded RNA having sequence complementary to the antisense strand of the siRNA duplex. Cleavage of the target RNA takes place in the middle of the region complementary to the antisense strand of the siRNA duplex (Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188).

RNAi has been studied in a variety of systems. Fire *et al.*, 1998, *Nature*, 391, 806, were the first to observe RNAi in *C. elegans*. Bahramian and Zarbl, 1999, *Molecular*

and *Cellular Biology*, 19, 274-283 and Wianny and Goetz, 1999, *Nature Cell Biol.*, 2, 70, describe RNAi mediated by dsRNA in mammalian systems. Hammond *et al.*, 2000, *Nature*, 404, 293, describe RNAi in *Drosophila* cells transfected with dsRNA. Elbashir *et al.*, 2001, *Nature*, 411, 494 and Tuschl *et al.*, International PCT Publication No. WO 01/75164, describe RNAi induced by introduction of duplexes of synthetic 21-nucleotide RNAs in cultured mammalian cells including human embryonic kidney and HeLa cells. Recent work in *Drosophila* embryonic lysates (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877 and Tuschl *et al.*, International PCT Publication No. WO 01/75164) has revealed certain requirements for siRNA length, structure, chemical composition, and sequence that are essential to mediate efficient RNAi activity. These studies have shown that 21-nucleotide siRNA duplexes are most active when containing 3'-terminal dinucleotide overhangs. Furthermore, complete substitution of one or both siRNA strands with 2'-deoxy (2'-H) or 2'-O-methyl nucleotides abolishes RNAi activity, whereas substitution of the 3'-terminal siRNA overhang nucleotides with 2'-deoxy nucleotides (2'-H) was shown to be tolerated. Single mismatch sequences in the center of the siRNA duplex were also shown to abolish RNAi activity. In addition, these studies also indicate that the position of the cleavage site in the target RNA is defined by the 5'-end of the siRNA guide sequence rather than the 3'-end of the guide sequence (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877). Other studies have indicated that a 5'-phosphate on the target-complementary strand of a siRNA duplex is required for siRNA activity and that ATP is utilized to maintain the 5'-phosphate moiety on the siRNA (Nykanen *et al.*, 2001, *Cell*, 107, 309).

Studies have shown that replacing the 3'-terminal nucleotide overhanging segments of a 21-mer siRNA duplex having two-nucleotide 3'-overhangs with deoxyribonucleotides does not have an adverse effect on RNAi activity. Replacing up to four nucleotides on each end of the siRNA with deoxyribonucleotides has been reported to be well tolerated, whereas complete substitution with deoxyribonucleotides results in no RNAi activity (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877 and Tuschl *et al.*, International PCT Publication No. WO 01/75164). In addition, Elbashir *et al.*, *supra*, also report that substitution of siRNA with 2'-O-methyl nucleotides completely abolishes RNAi activity. Li *et al.*, International PCT Publication No. WO 00/44914, and Beach *et al.*, International PCT Publication No. WO 01/68836 preliminarily suggest that siRNA may include modifications to either the phosphate-sugar backbone or the nucleoside to

include at least one of a nitrogen or sulfur heteroatom, however, neither application postulates to what extent such modifications would be tolerated in siRNA molecules, nor provides any further guidance or examples of such modified siRNA. Kreutzer *et al.*, Canadian Patent Application No. 2,359,180, also describe certain chemical modifications
5 for use in dsRNA constructs in order to counteract activation of double-stranded RNA-dependent protein kinase PKR, specifically 2'-amino or 2'-O-methyl nucleotides, and nucleotides containing a 2'-O or 4'-C methylene bridge. However, Kreutzer *et al.* similarly fails to provide examples or guidance as to what extent these modifications would be tolerated in dsRNA molecules.

10 Parrish *et al.*, 2000, *Molecular Cell*, 6, 1077-1087, tested certain chemical modifications targeting the unc-22 gene in *C. elegans* using long (>25 nt) siRNA transcripts. The authors describe the introduction of thiophosphate residues into these siRNA transcripts by incorporating thiophosphate nucleotide analogs with T7 and T3 RNA polymerase and observed that RNAs with two phosphorothioate modified bases
15 also had substantial decreases in effectiveness as RNAi. Further, Parrish *et al.* reported that phosphorothioate modification of more than two residues greatly destabilized the RNAs *in vitro* such that interference activities could not be assayed. *Id.* at 1081. The authors also tested certain modifications at the 2'-position of the nucleotide sugar in the long siRNA transcripts and found that substituting deoxynucleotides for ribonucleotides
20 produced a substantial decrease in interference activity, especially in the case of Uridine to Thymidine and/or Cytidine to deoxy-Cytidine substitutions. *Id.* In addition, the authors tested certain base modifications, including substituting, in sense and antisense strands of the siRNA, 4-thiouracil, 5-bromouracil, 5-iodouracil, and 3-(aminoallyl)uracil for uracil, and inosine for guanosine. Whereas 4-thiouracil and 5-bromouracil
25 substitution appeared to be tolerated, Parrish reported that inosine produced a substantial decrease in interference activity when incorporated in either strand. Parrish also reported that incorporation of 5-iodouracil and 3-(aminoallyl)uracil in the antisense strand resulted in a substantial decrease in RNAi activity as well.

The use of longer dsRNA has been described. For example, Beach *et al.*,
30 International PCT Publication No. WO 01/68836, describes specific methods for attenuating gene expression using endogenously-derived dsRNA. Tuschl *et al.*,

International PCT Publication No. WO 01/75164, describe a *Drosophila in vitro* RNAi system and the use of specific siRNA molecules for certain functional genomic and certain therapeutic applications; although Tuschl, 2001, *Chem. Biochem.*, 2, 239-245, doubts that RNAi can be used to cure genetic diseases or viral infection due to the danger of activating interferon response. Li *et al.*, International PCT Publication No. WO 00/44914, describe the use of specific long (141 bp-488 bp) enzymatically synthesized or vector expressed dsRNAs for attenuating the expression of certain target genes. Zernicka-Goetz *et al.*, International PCT Publication No. WO 01/36646, describe certain methods for inhibiting the expression of particular genes in mammalian cells using certain long (550 bp-714 bp), enzymatically synthesized or vector expressed dsRNA molecules. Fire *et al.*, International PCT Publication No. WO 99/32619, describe particular methods for introducing certain long dsRNA molecules into cells for use in inhibiting gene expression in nematodes. Plaetinck *et al.*, International PCT Publication No. WO 00/01846, describe certain methods for identifying specific genes responsible for conferring a particular phenotype in a cell using specific long dsRNA molecules. Mello *et al.*, International PCT Publication No. WO 01/29058, describe the identification of specific genes involved in dsRNA-mediated RNAi. Pachuck *et al.*, International PCT Publication No. WO 00/63364, describe certain long (at least 200 nucleotide) dsRNA constructs. Deschamps Depaillette *et al.*, International PCT Publication No. WO 99/07409, describe specific compositions consisting of particular dsRNA molecules combined with certain anti-viral agents. Waterhouse *et al.*, International PCT Publication No. 99/53050 and 1998, *PNAS*, 95, 13959-13964, describe certain methods for decreasing the phenotypic expression of a nucleic acid in plant cells using certain dsRNAs. Driscoll *et al.*, International PCT Publication No. WO 01/49844, describe specific DNA expression constructs for use in facilitating gene silencing in targeted organisms.

Others have reported on various RNAi and gene-silencing systems. For example, Parrish *et al.*, 2000, *Molecular Cell*, 6, 1077-1087, describe specific chemically-modified dsRNA constructs targeting the unc-22 gene of *C. elegans*. Grossniklaus, International PCT Publication No. WO 01/38551, describes certain methods for regulating polycomb gene expression in plants using certain dsRNAs. Churikov *et al.*, International PCT Publication No. WO 01/42443, describe certain methods for modifying genetic

characteristics of an organism using certain dsRNAs. Cogoni *et al.*, International PCT Publication No. WO 01/53475, describe certain methods for isolating a *Neurospora* silencing gene and uses thereof. Reed *et al.*, International PCT Publication No. WO 01/68836, describe certain methods for gene silencing in plants. Honer *et al.*,
5 International PCT Publication No. WO 01/70944, describe certain methods of drug screening using transgenic nematodes as Parkinson's Disease models using certain dsRNAs. Deak *et al.*, International PCT Publication No. WO 01/72774, describe certain *Drosophila*-derived gene products that may be related to RNAi in *Drosophila*. Arndt *et al.*, International PCT Publication No. WO 01/92513 describe certain methods for
10 mediating gene suppression by using factors that enhance RNAi. Tuschl *et al.*, International PCT Publication No. WO 02/44321, describe certain synthetic siRNA constructs. Pachuk *et al.*, International PCT Publication No. WO 00/63364, and Satishchandran *et al.*, International PCT Publication No. WO 01/04313, describe certain methods and compositions for inhibiting the function of certain polynucleotide
15 sequences using certain long (over 250 bp), vector expressed dsRNAs. Echeverri *et al.*, International PCT Publication No. WO 02/38805, describe certain *C. elegans* genes identified via RNAi. Kreutzer *et al.*, International PCT Publications Nos. WO 02/055692, WO 02/055693, and EP 1144623 B1 describes certain methods for inhibiting gene expression using dsRNA. Graham *et al.*, International PCT Publications Nos. WO
20 99/49029 and WO 01/70949, and AU 4037501 describe certain vector expressed siRNA molecules. Fire *et al.*, US 6,506,559, describe certain methods for inhibiting gene expression in vitro using certain long dsRNA (299 bp-1033 bp) constructs that mediate RNAi. Martinez *et al.*, 2002, *Cell*, 110, 563-574, describe certain single stranded siRNA constructs, including certain 5'-phosphorylated single stranded siRNAs that mediate
25 RNA interference in HeLa cells. Harborth *et al.*, 2003, *Antisense & Nucleic Acid Drug Development*, 13, 83-105, describe certain chemically and structurally modified siRNA molecules. Chiu and Rana, 2003, *RNA*, 9, 1034-1048, describe certain chemically and structurally modified siRNA molecules. Woolf *et al.*, International PCT Publication Nos. WO 03/064626 and WO 03/064625 describe certain chemically modified dsRNA
30 constructs.

SUMMARY OF THE INVENTION

This invention relates to compounds, compositions, and methods useful for modulating the expression of genes, such as those genes associated with neurodegenerative diseases, disorders, or conditions, such as Parkinson's disease, using short interfering nucleic acid (siNA) molecules. This invention also relates to compounds, compositions, and methods useful for modulating the expression and activity of genes associated with Parkinson's disease, such as PARK1 (SNCA), PARK2, PARK7, and PARK5, collectively "PARK genes" (see for example Dawson and Dawson, 2003, *J. Clin. Invest.*, 111, 145-151), ligands or receptors of PARK genes, or genes involved in PARK pathways of gene expression and/or activity by RNA interference (RNAi) using small nucleic acid molecules. In particular, the instant invention features small nucleic acid molecules, such as short interfering nucleic acid (siNA), short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules and methods used to modulate the expression of PARK genes (e.g., PARK1, PARK2, PARK7, and PARK5), particularly PARK genes, mutant PARK genes, or gene products associated with the development and/or maintenance of neurodegenerative diseases, disorders, and conditions such as Parkinson's disease.

A siNA of the invention can be unmodified or chemically-modified. A siNA of the instant invention can be chemically synthesized, expressed from a vector or enzymatically synthesized. The instant invention also features various chemically-modified synthetic short interfering nucleic acid (siNA) molecules capable of modulating PARK (e.g., PARK1, PARK2, PARK7, and PARK5) gene expression or activity in cells by RNA interference (RNAi). The use of chemically-modified siNA improves various properties of native siNA molecules through increased resistance to nuclease degradation *in vivo* and/or through improved cellular uptake. Further, contrary to earlier published studies, siNA having multiple chemical modifications retains its RNAi activity. The siNA molecules of the instant invention provide useful reagents and methods for a variety of therapeutic, diagnostic, target validation, genomic discovery, genetic engineering, and pharmacogenomic applications.

In one embodiment, the invention features one or more siNA molecules and methods that independently or in combination modulate the expression of Parkinson (PARK) gene(s), such as PARK1, PARK2, PARK7, and/or PARK5, associated with the maintenance and/or development of Parkinson's disease and other neurodegenerative diseases, such as genes encoding sequences comprising those sequences referred to by GenBank Accession Nos. shown in Table I, referred to herein generally as PARK. The description below of the various aspects and embodiments of the invention is provided with reference to the exemplary alpha-synuclein gene, generally referred to herein as SNCA (also known as PARK1). However, such reference is meant to be exemplary only and the various aspects and embodiments of the invention are also directed to other PARK genes (e.g., PARK1, PARK2, PARK7, and/or PARK5). For example, the various aspects and embodiments of the invention are also directed the modulation of alternate PARK genes, such as PARK homolog genes, transcript variants, and polymorphisms (e.g., single nucleotide polymorphism, (SNPs)) associated with certain PARK genes. such as other PARK gene isoforms (e.g., alpha-synuclein, beta-synuclein, gamma-synuclein), mutant PARK genes (e.g., mutant versions of SNCA), splice variants of PARK genes, and genes encoding any ligands or receptors of PARK gene products. The various aspects and embodiments are also directed to other genes that are involved in PARK gene (e.g., SNCA) mediated pathways of signal transduction or gene expression that are involved in the progression, development, and/or maintenance of disease (e.g., Parkinson's disease). These additional genes can be analyzed for target sites using the methods described for SNCA genes herein. Thus, the modulation of other genes and the effects of such modulation of the other genes can be performed, determined, and measured as described herein.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a PARK (e.g., PARK1, PARK2, PARK5, and/or PARK7) gene, wherein said siNA molecule comprises about 19 to about 21 base pairs.

In one embodiment, the invention features a siNA molecule that down-regulates expression of a PARK (e.g., PARK1, PARK2, PARK5, and/or PARK7) gene, for

example, wherein the PARK gene comprises PARK1, PARK2, PARK5, and/or PARK7 or mutant PARK1, PARK2, PARK5, and/or PARK7 encoding sequence.

5 In one embodiment, the invention features a siNA molecule having RNAi activity against PARK1, PARK2, PARK5, and/or PARK7 RNA, wherein the siNA molecule comprises a sequence complementary to any RNA having PARK1, PARK2, PARK5, and/or PARK7 encoding sequence, including, but not limited to those sequences having GenBank Accession Nos. shown in Table I. In another embodiment, the invention features a siNA molecule having RNAi activity against PARK1, PARK2, PARK5, and/or PARK7 RNA, wherein the siNA molecule comprises a sequence complementary to an RNA having other PARK1, PARK2, PARK5, and/or PARK7 encoding sequence, for example, mutant PARK1, PARK2, PARK5, and/or PARK7 genes, splice variants of PARK1, PARK2, PARK5, and/or PARK7 genes, variants of PARK1, PARK2, PARK5, and/or PARK7 genes with conservative substitutions, and homologous PARK1, PARK2, PARK5, and/or PARK7 ligands and receptors and the like. Chemical modifications as shown in Tables III and IV or otherwise described herein or elsewhere in the art can readily be applied to any siNA construct of the invention.

20 In one embodiment, a siNA of the invention is used to inhibit the expression of PARK1, PARK2, PARK5, and/or PARK7 genes or a PARK1, PARK2, PARK5, and/or PARK7 gene family, wherein the genes or gene family sequences share sequence homology. Such homologous sequences can be identified as is known in the art, for example using sequence alignments. siNA molecules can be designed to target such homologous sequences, for example using perfectly complementary sequences or by incorporating non-canonical base pairs, for example mismatches and/or wobble base pairs, that can provide additional target sequences. In instances where mismatches are identified, non-canonical base pairs, for example mismatches and/or wobble bases, can be used to generate siNA molecules that target both more than one gene sequences. In a non-limiting example, non-canonical base pairs such as UU and CC base pairs are used to generate siNA molecules that are capable of targeting sequences for differing PARK1, PARK2, PARK5, and/or PARK7 targets that share sequence homology (e.g., differing allelic variants). As such, one advantage of using siNAs of the invention is that a single siNA can be designed to include nucleic acid sequence that is complementary to the

nucleotide sequence that is conserved between the homologous genes. In this approach, a single siNA can be used to inhibit expression of more than one gene instead of using more than one siNA molecule to target the different genes.

In one embodiment, the invention features a siNA molecule having RNAi activity
5 against PARK1, PARK2, PARK5, and/or PARK7 RNA, wherein the siNA molecule
comprises a sequence complementary to any RNA having PARK1, PARK2, PARK5,
and/or PARK7 encoding sequence, such as those sequences having GenBank Accession
Nos. shown in Table I. In another embodiment, the invention features a siNA molecule
having RNAi activity against PARK1, PARK2, PARK5, and/or PARK7 RNA, wherein
10 the siNA molecule comprises a sequence complementary to an RNA having variant
PARK1, PARK2, PARK5, and/or PARK7 encoding sequence, for example other mutant
PARK1, PARK2, PARK5, and/or PARK7 genes not shown in Table I but known in the
art to be associated with the maintenance and/or development of Parkinson's disease,
Alzheimer's disease, and/or dementia. Chemical modifications as shown in Tables III
15 and IV or otherwise described herein can be applied to any siNA construct of the
invention. In another embodiment, a siNA molecule of the invention includes a
nucleotide sequence that can interact with nucleotide sequence of a PARK1, PARK2,
PARK5, and/or PARK7 gene and thereby mediate silencing of PARK1, PARK2,
PARK5, and/or PARK7 gene expression, for example, wherein the siNA mediates
20 regulation of PARK1, PARK2, PARK5, and/or PARK7 gene expression by cellular
processes that modulate the chromatin structure or methylation patterns of the PARK1,
PARK2, PARK5, and/or PARK7 gene and prevent transcription of the PARK1, PARK2,
PARK5, and/or PARK7 gene.

In one embodiment, siNA molecules of the invention are used to down regulate or
25 inhibit the expression of PARK1, PARK2, PARK5, and/or PARK7 proteins arising from
PARK1, PARK2, PARK5, and/or PARK7 haplotype polymorphisms that are associated
with a disease or condition, (e.g., Parkinson's disease, Alzheimer's disease, and/or
dementia). Analysis of PARK1, PARK2, PARK5, and/or PARK7 genes, or PARK1,
PARK2, PARK5, and/or PARK7 protein or RNA levels can be used to identify subjects
30 with such polymorphisms or those subjects who are at risk of developing traits,
conditions, or diseases described herein. These subjects are amenable to treatment, for

example, treatment with siNA molecules of the invention and any other composition useful in treating diseases related to PARK1, PARK2, PARK5, and/or PARK7 gene expression. As such, analysis of PARK1, PARK2, PARK5, and/or PARK7 protein or RNA levels can be used to determine treatment type and the course of therapy in treating
5 a subject. Monitoring of PARK1, PARK2, PARK5, and/or PARK7 protein or RNA levels can be used to predict treatment outcome and to determine the efficacy of compounds and compositions that modulate the level and/or activity of certain PARK1, PARK2, PARK5, and/or PARK7 proteins associated with a trait, condition, or disease.

In one embodiment of the invention a siNA molecule comprises an antisense
10 strand comprising a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof encoding a PARK1, PARK2, PARK5, and/or PARK7 protein. The siNA further comprises a sense strand, wherein said sense strand comprises a nucleotide sequence of a PARK1, PARK2, PARK5, and/or PARK7 gene or a portion thereof.

In another embodiment, the invention features a siNA molecule comprising a
15 nucleotide sequence in the antisense region of the siNA molecule that is complementary to a nucleotide sequence or portion of sequence of a PARK1, PARK2, PARK5, and/or PARK7 gene. In another embodiment, the invention features a siNA molecule comprising a region, for example, the antisense region of the siNA construct, complementary to a sequence comprising a PARK1, PARK2, PARK5, and/or PARK7
20 gene sequence or a portion thereof.

In one embodiment, the antisense region of SNCA siNA constructs can comprise a sequence complementary to sequence having any of SEQ ID NOs. 1-86 or 173-210. In one embodiment, the antisense region can also comprise sequence having any of SEQ ID NOs. 87-172, 211-248, 257-260, 265-268, 273-276, 281-284, 289-292, 297-304, 313-
25 320, 329-352, 354, 356, 358, 361, 363, 365, 367, or 370. In another embodiment, the sense region of the SNCA constructs can comprise sequence having any of SEQ ID NOs. 1-86, 173-210, 249-252, 253-256, 261-264, 269-272, 277-280, 285-288, 293-296, 305-312, 321-328, 353, 355, 357, 359, 360, 362, 364, 366, 368, or 369.

In one embodiment, a siNA molecule of the invention comprises any of SEQ ID
30 NOs. 1-370. The sequences shown in SEQ ID NOs: 1-370 are not limiting. A siNA

molecule of the invention can comprise any contiguous SNCA sequence (e.g., about 19 to about 25, or about 19, 20, 21, 22, 23, 24 or 25 contiguous SNCA nucleotides).

In yet another embodiment, the invention features a siNA molecule comprising a sequence, for example, the antisense sequence of the siNA construct, complementary to a sequence or portion of sequence comprising sequence represented by GenBank
5 Accession Nos. shown in Table I. Chemical modifications in Tables III and IV and described herein can be applied to any siNA construct of the invention.

In one embodiment of the invention a siNA molecule comprises an antisense strand having about 19 to about 29 (e.g., about 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or
10 29) nucleotides, wherein the antisense strand is complementary to a RNA sequence encoding a PARK1, PARK2, PARK5, and/or PARK7 protein, and wherein said siNA further comprises a sense strand having about 19 to about 29 (e.g., about 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) nucleotides, and wherein said sense strand and said antisense strand are distinct nucleotide sequences with at least about 19 complementary
15 nucleotides.

In another embodiment of the invention a siNA molecule of the invention comprises an antisense region having about 19 to about 29 (e.g., about 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) nucleotides, wherein the antisense region is complementary to a RNA sequence encoding a PARK1, PARK2, PARK5, and/or PARK7 protein, and
20 wherein said siNA further comprises a sense region having about 19 to about 29 (e.g., about 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) nucleotides, wherein said sense region and said antisense region comprise a linear molecule with at least about 19 complementary nucleotides.

In one embodiment, a siNA molecule of the invention has RNAi activity that
25 modulates expression of RNA encoded by a PARK1, PARK2, PARK5, and/or PARK7 gene. Because synuclein genes (e.g., PARK1, PARK2, PARK5, and/or PARK7 and mutant or polymorphic versions thereof) can share some degree of sequence homology with each other, siNA molecules can be designed to target a class of synuclein genes (and associated receptor or ligand genes) or alternately specific synuclein genes by
30 selecting sequences that are either shared amongst different synuclein targets or

alternatively that are unique for a specific synuclein target. Therefore, in one embodiment, the siNA molecule can be designed to target conserved regions of PARK1, PARK2, PARK5, and/or PARK7 RNA sequences having homology among several PARK1, PARK2, PARK5, and/or PARK7 gene variants so as to target a class of
5 PARK1, PARK2, PARK5, and/or PARK7 genes with one siNA molecule. Accordingly, in one embodiment, the siNA molecule of the invention modulates the expression of one or both PARK1, PARK2, PARK5, and/or PARK7 alleles in a subject. In another embodiment, the siNA molecule can be designed to target a sequence that is unique to a specific PARK1, PARK2, PARK5, and/or PARK7 RNA sequence (e.g., a single
10 PARK1, PARK2, PARK5, and/or PARK7 allele or PARK1, PARK2, PARK5, and/or PARK7 allele, mutation, or single nucleotide polymorphism (SNP)) due to the high degree of specificity that the siNA molecule requires to mediate RNAi activity.

In one embodiment, nucleic acid molecules of the invention that act as mediators of the RNA interference gene silencing response are double-stranded nucleic acid
15 molecules. In another embodiment, the siNA molecules of the invention consist of duplex nucleic acid molecules containing about 19 base pairs between oligonucleotides comprising about 19 to about 25 (e.g., about 19, 20, 21, 22, 23, 24, or 25) nucleotides. In yet another embodiment, siNA molecules of the invention comprise duplex nucleic acid molecules with overhanging ends of about about 1 to about 3 (e.g., about 1, 2, or 3)
20 nucleotides, for example, about 21-nucleotide duplexes with about 19 base pairs and 3'-terminal mononucleotide, dinucleotide, or trinucleotide overhangs.

In one embodiment, a siNA molecule of the invention targeting PARK1, PARK2, PARK5, and/or PARK7 is used in combination with another neuroprotective therapy or compound. Such neuroprotective therapies and compounds can include compositions
25 that modulate the expression or activity of beta amyloid or amyloid precursor protein. For example, a siNA molecule of the invention targeting PARK1, PARK2, PARK5, and/or PARK7 is used in combination with one or more siNA molecules targeting beta-secretase, as described in McSwiggen *et al.*, PCT/US03/04710 filed February 18, 2003 and USSN 10/607,933, filed June 27, 2003, both incorporated by reference herein in
30 their entirety including the drawings.

In one embodiment, the invention features one or more chemically-modified siNA constructs having specificity for PARK1, PARK2, PARK5, and/or PARK7 expressing nucleic acid molecules, such as RNA encoding a PARK1, PARK2, PARK5, and/or PARK7 protein. Non-limiting examples of such chemical modifications include without
5 limitation phosphorothioate internucleotide linkages, 2'-deoxyribonucleotides, 2'-O-methyl ribonucleotides, 2'-deoxy-2'-fluoro ribonucleotides, "universal base" nucleotides, "acyclic" nucleotides, 5-C-methyl nucleotides, and terminal glyceryl and/or inverted deoxy abasic residue incorporation. These chemical modifications, when used in various siNA constructs, are shown to preserve RNAi activity in cells while at the same time,
10 dramatically increasing the serum stability of these compounds. Furthermore, contrary to the data published by Parrish *et al.*, *supra*, applicant demonstrates that multiple (greater than one) phosphorothioate substitutions are well-tolerated and confer substantial increases in serum stability for modified siNA constructs.

In one embodiment, a siNA molecule of the invention comprises modified
15 nucleotides while maintaining the ability to mediate RNAi. The modified nucleotides can be used to improve *in vitro* or *in vivo* characteristics such as stability, activity, and/or bioavailability. For example, a siNA molecule of the invention can comprise modified nucleotides as a percentage of the total number of nucleotides present in the siNA molecule. As such, a siNA molecule of the invention can generally comprise about 5%
20 to about 100% modified nucleotides (e.g., about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% modified nucleotides). The actual percentage of modified nucleotides present in a given siNA molecule will depend on the total number of nucleotides present in the siNA. If the siNA molecule is single stranded, the percent modification can be based upon the total number
25 of nucleotides present in the single stranded siNA molecules. Likewise, if the siNA molecule is double stranded, the percent modification can be based upon the total number of nucleotides present in the sense strand, antisense strand, or both the sense and antisense strands.

One aspect of the invention features a double-stranded short interfering nucleic
30 acid (siNA) molecule that down-regulates expression of a PARK1, PARK2, PARK5, and/or PARK7 gene. In one embodiment, the double stranded siNA molecule comprises

one or more chemical modifications and each strand of the double-stranded siNA is about 21 nucleotides long. In one embodiment, the double-stranded siNA molecule does not contain any ribonucleotides. In another embodiment, the double-stranded siNA molecule comprises one or more ribonucleotides. In one embodiment, each strand of the double-stranded siNA molecule comprises about 19 to about 29 (e.g., about 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) nucleotides, wherein each strand comprises about 19 nucleotides that are complementary to the nucleotides of the other strand. In one embodiment, one of the strands of the double-stranded siNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of the PARK1, PARK2, PARK5, and/or PARK7 gene, and the second strand of the double-stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence of the PARK1, PARK2, PARK5, and/or PARK7 gene or a portion thereof.

In another embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a PARK1, PARK2, PARK5, and/or PARK7 gene comprising an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of the PARK1, PARK2, PARK5, and/or PARK7 gene or a portion thereof, and a sense region, wherein the sense region comprises a nucleotide sequence substantially similar to the nucleotide sequence of the PARK1, PARK2, PARK5, and/or PARK7 gene or a portion thereof. In one embodiment, the antisense region and the sense region each comprise about 19 to about 23 (e.g. about 19, 20, 21, 22, or 23) nucleotides, wherein the antisense region comprises about 19 nucleotides that are complementary to nucleotides of the sense region.

In another embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a PARK1, PARK2, PARK5, and/or PARK7 gene comprising a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA encoded by the PARK1, PARK2, PARK5, and/or PARK7 gene or a portion thereof and the sense region comprises a nucleotide sequence that is complementary to the antisense region.

In one embodiment, a siNA molecule of the invention comprises blunt ends, i.e., ends that do not include any overhanging nucleotides. For example, a siNA molecule comprising modifications described herein (e.g., comprising nucleotides having Formulae I-VII or siNA constructs comprising "Stab 00"- "Stab 25" (Table IV) or any combination thereof (see Table IV)) and/or any length described herein can comprise blunt ends or ends with no overhanging nucleotides.

In one embodiment, any siNA molecule of the invention can comprise one or more blunt ends, i.e. where a blunt end does not have any overhanging nucleotides. In one embodiment, the blunt ended siNA molecule has a number of base pairs equal to the number of nucleotides present in each strand of the siNA molecule. In another embodiment, the siNA molecule comprises one blunt end, for example wherein the 5'-end of the antisense strand and the 3'-end of the sense strand do not have any overhanging nucleotides. In another example, the siNA molecule comprises one blunt end, for example wherein the 3'-end of the antisense strand and the 5'-end of the sense strand do not have any overhanging nucleotides. In another example, a siNA molecule comprises two blunt ends, for example wherein the 3'-end of the antisense strand and the 5'-end of the sense strand as well as the 5'-end of the antisense strand and 3'-end of the sense strand do not have any overhanging nucleotides. A blunt ended siNA molecule can comprise, for example, from about 18 to about 30 nucleotides (e.g., about 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides). Other nucleotides present in a blunt ended siNA molecule can comprise mismatches, bulges, loops, or wobble base pairs, for example, to modulate the activity of the siNA molecule to mediate RNA interference.

By "blunt ends" is meant symmetric termini or termini of a double stranded siNA molecule having no overhanging nucleotides. The two strands of a double stranded siNA molecule align with each other without over-hanging nucleotides at the termini. For example, a blunt ended siNA construct comprises terminal nucleotides that are complementary between the sense and antisense regions of the siNA molecule.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a PARK1, PARK2, PARK5, and/or PARK7 gene, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and

the second fragment comprises the antisense region of the siNA molecule. The sense region can be connected to the antisense region via a linker molecule, such as a polynucleotide linker or a non-nucleotide linker.

In one embodiment, the invention features double-stranded short interfering
5 nucleic acid (siNA) molecule that down-regulates expression of a PARK1, PARK2, PARK5, and/or PARK7 gene, wherein the siNA molecule comprises about 19 to about 21 base pairs, and wherein each strand of the siNA molecule comprises one or more chemical modifications. In another embodiment, one of the strands of the double-stranded siNA molecule comprises a nucleotide sequence that is complementary to a
10 nucleotide sequence of a PARK1, PARK2, PARK5, and/or PARK7 gene or a portion thereof, and the second strand of the double-stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence or a portion thereof of the PARK1, PARK2, PARK5, and/or PARK7 gene. In another embodiment, one of the strands of the double-stranded siNA molecule comprises a nucleotide sequence that
15 is complementary to a nucleotide sequence of a PARK1, PARK2, PARK5, and/or PARK7 gene or portion thereof, and the second strand of the double-stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence or portion thereof of the PARK1, PARK2, PARK5, and/or PARK7 gene. In another embodiment, each strand of the siNA molecule comprises about 19 to about 23
20 nucleotides, and each strand comprises at least about 19 nucleotides that are complementary to the nucleotides of the other strand. The PARK1, PARK2, PARK5, and/or PARK7 gene can comprise, for example, sequences referred to in Table I.

In one embodiment, a siNA molecule of the invention comprises no ribonucleotides. In another embodiment, a siNA molecule of the invention comprises
25 ribonucleotides.

In one embodiment, a siNA molecule of the invention comprises an antisense region comprising a nucleotide sequence that is complementary to a nucleotide sequence of a PARK1, PARK2, PARK5, and/or PARK7 gene or a portion thereof, and the siNA further comprises a sense region comprising a nucleotide sequence substantially similar
30 to the nucleotide sequence of the PARK1, PARK2, PARK5, and/or PARK7 gene or a portion thereof. In another embodiment, the antisense region and the sense region each

comprise about 19 to about 23 nucleotides and the antisense region comprises at least about 19 nucleotides that are complementary to nucleotides of the sense region. The PARK1, PARK2, PARK5, and/or PARK7 gene can comprise, for example, sequences referred to in Table I.

5 In one embodiment, a siNA molecule of the invention comprises a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA encoded by a PARK1, PARK2, PARK5, and/or PARK7 gene, or a portion thereof, and the sense region comprises a nucleotide sequence that is complementary to the antisense region. In one embodiment,
10 the siNA molecule is assembled from two separate oligonucleotide fragments, wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule. In another embodiment, the sense region is connected to the antisense region via a linker molecule. In another embodiment, the sense region is connected to the antisense region via a linker molecule, such as a
15 nucleotide or non-nucleotide linker. The PARK1, PARK2, PARK5, and/or PARK7 gene can comprise, for example, sequences referred in to Table I.

 In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a PARK1, PARK2, PARK5, and/or PARK7 gene comprising a sense region and an antisense region, wherein
20 the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA encoded by the PARK1, PARK2, PARK5, and/or PARK7 gene or a portion thereof and the sense region comprises a nucleotide sequence that is complementary to the antisense region, and wherein the siNA molecule has one or more modified pyrimidine and/or purine nucleotides. In one embodiment, the pyrimidine
25 nucleotides in the sense region are 2'-O-methyl pyrimidine nucleotides or 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-deoxy purine nucleotides. In another embodiment, the pyrimidine nucleotides in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-O-methyl purine nucleotides. In another embodiment,
30 the pyrimidine nucleotides in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-deoxy purine

nucleotides. In one embodiment, the pyrimidine nucleotides in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the antisense region are 2'-O-methyl or 2'-deoxy purine nucleotides. In another embodiment of any of the above-described siNA molecules, any nucleotides present in a non-complementary region of the sense strand (e.g. overhang region) are 2'-deoxy nucleotides.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a PARK1, PARK2, PARK5, and/or PARK7 gene, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule, and wherein the fragment comprising the sense region includes a terminal cap moiety at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the fragment. In one embodiment, the terminal cap moiety is an inverted deoxy abasic moiety or glyceryl moiety. In one embodiment, each of the two fragments of the siNA molecule comprise about 21 nucleotides.

In one embodiment, the invention features a siNA molecule comprising at least one modified nucleotide, wherein the modified nucleotide is a 2'-deoxy-2'-fluoro nucleotide. The siNA can be, for example, of length between about 12 and about 36 nucleotides. In one embodiment, all pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides. In one embodiment, the modified nucleotides in the siNA include at least one 2'-deoxy-2'-fluoro cytidine or 2'-deoxy-2'-fluoro uridine nucleotide. In another embodiment, the modified nucleotides in the siNA include at least one 2'-fluoro cytidine and at least one 2'-deoxy-2'-fluoro uridine nucleotides. In one embodiment, all uridine nucleotides present in the siNA are 2'-deoxy-2'-fluoro uridine nucleotides. In one embodiment, all cytidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro cytidine nucleotides. In one embodiment, all adenosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro adenosine nucleotides. In one embodiment, all guanosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro guanosine nucleotides. The siNA can further comprise at least one modified internucleotidic linkage, such as phosphorothioate linkage. In one embodiment, the 2'-deoxy-2'-

fluronucleotides are present at specifically selected locations in the siNA that are sensitive to cleavage by ribonucleases, such as locations having pyrimidine nucleotides.

In one embodiment, the invention features a method of increasing the stability of a siNA molecule against cleavage by ribonucleases comprising introducing at least one modified nucleotide into the siNA molecule, wherein the modified nucleotide is a 2'-deoxy-2'-fluoro nucleotide. In one embodiment, all pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides. In one embodiment, the modified nucleotides in the siNA include at least one 2'-deoxy-2'-fluoro cytidine or 2'-deoxy-2'-fluoro uridine nucleotide. In another embodiment, the modified nucleotides in the siNA include at least one 2'-fluoro cytidine and at least one 2'-deoxy-2'-fluoro uridine nucleotides. In one embodiment, all uridine nucleotides present in the siNA are 2'-deoxy-2'-fluoro uridine nucleotides. In one embodiment, all cytidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro cytidine nucleotides. In one embodiment, all adenosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro adenosine nucleotides. In one embodiment, all guanosine nucleotides present in the siNA are 2'-deoxy-2'-fluoro guanosine nucleotides. The siNA can further comprise at least one modified internucleotidic linkage, such as phosphorothioate linkage. In one embodiment, the 2'-deoxy-2'-fluronucleotides are present at specifically selected locations in the siNA that are sensitive to cleavage by ribonucleases, such as locations having pyrimidine nucleotides.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a PARK1, PARK2, PARK5, and/or PARK7 gene comprising a sense region and an antisense region, wherein the antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA encoded by the PARK1, PARK2, PARK5, and/or PARK7 gene or a portion thereof and the sense region comprises a nucleotide sequence that is complementary to the antisense region, and wherein the purine nucleotides present in the antisense region comprise 2'-deoxy- purine nucleotides. In an alternative embodiment, the purine nucleotides present in the antisense region comprise 2'-O-methyl purine nucleotides. In either of the above embodiments, the antisense region can comprise a phosphorothioate internucleotide linkage at the 3' end of the antisense region.

Alternatively, in either of the above embodiments, the antisense region can comprise a glyceryl modification at the 3' end of the antisense region. In another embodiment of any of the above-described siNA molecules, any nucleotides present in a non-complementary region of the antisense strand (e.g. overhang region) are 2'-deoxy nucleotides.

In one embodiment, the antisense region of a siNA molecule of the invention comprises sequence complementary to a portion of a PARK1, PARK2, PARK5, and/or PARK7 transcript having sequence unique to a particular PARK1, PARK2, PARK5, and/or PARK7 disease related allele, such as sequence comprising a single nucleotide polymorphism (SNP) associated with the disease specific allele. As such, the antisense region of a siNA molecule of the invention can comprise sequence complementary to sequences that are unique to a particular allele to provide specificity in mediating selective RNAi against the disease, condition, or trait related allele.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that down-regulates expression of a PARK1, PARK2, PARK5, and/or PARK7 gene, wherein the siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of the siNA molecule. In another embodiment about 19 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule and wherein at least two 3' terminal nucleotides of each fragment of the siNA molecule are not base-paired to the nucleotides of the other fragment of the siNA molecule. In one embodiment, each of the two 3' terminal nucleotides of each fragment of the siNA molecule is a 2'-deoxy-pyrimidine nucleotide, such as a 2'-deoxy-thymidine. In another embodiment, all 21 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule. In another embodiment, about 19 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the PARK1, PARK2, PARK5, and/or PARK7 gene. In another embodiment, about 21 nucleotides of the antisense region are base-paired to the nucleotide sequence or a portion thereof of the RNA encoded by the PARK1, PARK2, PARK5, and/or PARK7 gene. In any of the

above embodiments, the 5'-end of the fragment comprising said antisense region can optionally includes a phosphate group.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits the expression of a PARK1, PARK2, PARK5, and/or PARK7 RNA sequence (e.g., wherein said target RNA sequence is encoded by a PARK1, PARK2, PARK5, and/or PARK7 gene involved in the PARK1, PARK2, PARK5, and/or PARK7 pathway), wherein the siNA molecule does not contain any ribonucleotides and wherein each strand of the double-stranded siNA molecule is about 21 nucleotides long. Examples of non-ribonucleotide containing siNA constructs are combinations of stabilization chemistries shown in Table IV in any combination of Sense/Antisense chemistries, such as Stab 7/8, Stab 7/11, Stab 8/8, Stab 18/8, Stab 18/11, Stab 12/13, Stab 7/13, Stab 18/13, Stab 7/19, Stab 8/19, Stab 18/19, Stab 7/20, Stab 8/20, or Stab 18/20.

In one embodiment, the invention features a chemically synthesized double stranded RNA molecule that directs cleavage of a PARK1, PARK2, PARK5, and/or PARK7 RNA via RNA interference, wherein each strand of said RNA molecule is about 21 to about 23 nucleotides in length; one strand of the RNA molecule comprises nucleotide sequence having sufficient complementarity to the PARK1, PARK2, PARK5, and/or PARK7 RNA for the RNA molecule to direct cleavage of the PARK1, PARK2, PARK5, and/or PARK7 RNA via RNA interference; and wherein at least one strand of the RNA molecule comprises one or more chemically modified nucleotides described herein, such as deoxynucleotides, 2'-O-methyl nucleotides, 2'-deoxy-2'-fluoro nucleotides, 2'-O-methoxyethyl nucleotides etc.

In one embodiment, the invention features a medicament comprising a siNA molecule of the invention.

In one embodiment, the invention features an active ingredient comprising a siNA molecule of the invention.

In one embodiment, the invention features the use of a double-stranded short interfering nucleic acid (siNA) molecule to down-regulate expression of a PARK1,

PARK2, PARK5, and/or PARK7 gene, wherein the siNA molecule comprises one or more chemical modifications and each strand of the double-stranded siNA is about 18 to about 28 or more (e.g., about 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 or more) nucleotides long.

5 In one embodiment, the invention features the use of a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a PARK1, PARK2, PARK5, and/or PARK7 gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of PARK1, PARK2, PARK5, and/or PARK7
10 RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification.

 In one embodiment, the invention features a double-stranded short interfering
15 nucleic acid (siNA) molecule that inhibits expression of a PARK1, PARK2, PARK5, and/or PARK7 gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of PARK1, PARK2, PARK5, and/or PARK7 RNA or a portion thereof, wherein the other strand is a sense strand which comprises nucleotide sequence
20 that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification.

 In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a PARK1, PARK2, PARK5,
25 and/or PARK7 gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to nucleotide sequence of PARK1, PARK2, PARK5, and/or PARK7 RNA that encodes a protein or portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and
30 wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification. In one embodiment, each strand of the siNA

molecule comprises about 18 to about 29 or more (e.g., about 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29 or more) nucleotides, wherein each strand comprises at least about 18 nucleotides that are complementary to the nucleotides of the other strand. In one embodiment, the siNA molecule is assembled from two oligonucleotide fragments, wherein one fragment comprises the nucleotide sequence of the antisense strand of the siNA molecule and a second fragment comprises nucleotide sequence of the sense region of the siNA molecule. In one embodiment, the sense strand is connected to the antisense strand via a linker molecule, such as a polynucleotide linker or a non-nucleotide linker. In a further embodiment, the pyrimidine nucleotides present in the sense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-deoxy purine nucleotides. In another embodiment, the pyrimidine nucleotides present in the sense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and the purine nucleotides present in the sense region are 2'-O-methyl purine nucleotides. In still another embodiment, the pyrimidine nucleotides present in the antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and any purine nucleotides present in the antisense strand are 2'-deoxy purine nucleotides. In another embodiment, the antisense strand comprises one or more 2'-deoxy-2'-fluoro pyrimidine nucleotides and one or more 2'-O-methyl purine nucleotides. In another embodiment, the pyrimidine nucleotides present in the antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides and any purine nucleotides present in the antisense strand are 2'-O-methyl purine nucleotides. In a further embodiment the sense strand comprises a 3'-end and a 5'-end, wherein a terminal cap moiety (e.g., an inverted deoxy abasic moiety or inverted deoxy nucleotide moiety such as inverted thymidine) is present at the 5'-end, the 3'-end, or both of the 5' and 3' ends of the sense strand. In another embodiment, the antisense strand comprises a phosphorothioate internucleotide linkage at the 3' end of the antisense strand. In another embodiment, the antisense strand comprises a glyceryl modification at the 3' end. In another embodiment, the 5'-end of the antisense strand optionally includes a phosphate group.

In any of the above-described embodiments of a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a PARK1, PARK2, PARK5, and/or PARK7 gene, wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, each of the two strands

of the siNA molecule can comprise about 21 nucleotides. In one embodiment, about 21 nucleotides of each strand of the siNA molecule are base-paired to the complementary nucleotides of the other strand of the siNA molecule. In another embodiment, about 19 nucleotides of each strand of the siNA molecule are base-paired to the complementary
5 nucleotides of the other strand of the siNA molecule, wherein at least two 3' terminal nucleotides of each strand of the siNA molecule are not base-paired to the nucleotides of the other strand of the siNA molecule. In another embodiment, each of the two 3' terminal nucleotides of each fragment of the siNA molecule is a 2'-deoxy-pyrimidine, such as 2'-deoxy-thymidine. In one embodiment, each strand of the siNA molecule is
10 base-paired to the complementary nucleotides of the other strand of the siNA molecule. In one embodiment, about 19 nucleotides of the antisense strand are base-paired to the nucleotide sequence of the PARK1, PARK2, PARK5, and/or PARK7 RNA or a portion thereof. In one embodiment, about 21 nucleotides of the antisense strand are base-paired to the nucleotide sequence of the PARK1, PARK2, PARK5, and/or PARK7 RNA or a
15 portion thereof.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a PARK1, PARK2, PARK5, and/or PARK7 gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to
20 nucleotide sequence of PARK1, PARK2, PARK5, and/or PARK7 RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the 5'-end of the antisense strand optionally includes a
25 phosphate group.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a PARK1, PARK2, PARK5, and/or PARK7 gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to
30 nucleotide sequence of PARK1, PARK2, PARK5, and/or PARK7 RNA or a portion thereof, the other strand is a sense strand which comprises nucleotide sequence that is

complementary to a nucleotide sequence of the antisense strand and wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the nucleotide sequence or a portion thereof of the antisense strand is complementary to a nucleotide sequence of the untranslated region or
5 a portion thereof of the PARK1, PARK2, PARK5, and/or PARK7 RNA.

In one embodiment, the invention features a double-stranded short interfering nucleic acid (siNA) molecule that inhibits expression of a PARK1, PARK2, PARK5, and/or PARK7 gene, wherein one of the strands of the double-stranded siNA molecule is an antisense strand which comprises nucleotide sequence that is complementary to
10 nucleotide sequence of PARK1, PARK2, PARK5, and/or PARK7 RNA or a portion thereof, wherein the other strand is a sense strand which comprises nucleotide sequence that is complementary to a nucleotide sequence of the antisense strand, wherein a majority of the pyrimidine nucleotides present in the double-stranded siNA molecule comprises a sugar modification, and wherein the nucleotide sequence of the antisense
15 strand is complementary to a nucleotide sequence of the PARK1, PARK2, PARK5, and/or PARK7 RNA or a portion thereof that is present in the PARK1, PARK2, PARK5, and/or PARK7 RNA.

In one embodiment, the invention features a composition comprising a siNA molecule of the invention in a pharmaceutically acceptable carrier or diluent.

20 In a non-limiting example, the introduction of chemically-modified nucleotides into nucleic acid molecules provides a powerful tool in overcoming potential limitations of *in vivo* stability and bioavailability inherent to native RNA molecules that are delivered exogenously. For example, the use of chemically-modified nucleic acid molecules can enable a lower dose of a particular nucleic acid molecule for a given
25 therapeutic effect since chemically-modified nucleic acid molecules tend to have a longer half-life in serum. Furthermore, certain chemical modifications can improve the bioavailability of nucleic acid molecules by targeting particular cells or tissues and/or improving cellular uptake of the nucleic acid molecule. Therefore, even if the activity of a chemically-modified nucleic acid molecule is reduced as compared to a native nucleic
30 acid molecule, for example, when compared to an all-RNA nucleic acid molecule, the overall activity of the modified nucleic acid molecule can be greater than that of the

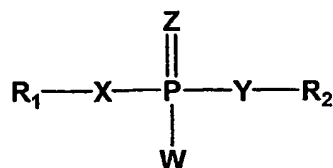
native molecule due to improved stability and/or delivery of the molecule. Unlike native unmodified siNA, chemically-modified siNA can also minimize the possibility of activating interferon activity in humans.

In any of the embodiments of siNA molecules described herein, the antisense
5 region of a siNA molecule of the invention can comprise a phosphorothioate internucleotide linkage at the 3'-end of said antisense region. In any of the embodiments of siNA molecules described herein, the antisense region can comprise about one to about five phosphorothioate internucleotide linkages at the 5'-end of said antisense region. In any of the embodiments of siNA molecules described herein, the 3'-terminal
10 nucleotide overhangs of a siNA molecule of the invention can comprise ribonucleotides or deoxyribonucleotides that are chemically-modified at a nucleic acid sugar, base, or backbone. In any of the embodiments of siNA molecules described herein, the 3'-terminal nucleotide overhangs can comprise one or more universal base ribonucleotides. In any of the embodiments of siNA molecules described herein, the 3'-terminal
15 nucleotide overhangs can comprise one or more acyclic nucleotides.

One embodiment of the invention provides an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the invention in a manner that allows expression of the nucleic acid molecule. Another embodiment of the invention provides a mammalian cell comprising such an expression vector. The
20 mammalian cell can be a human cell. The siNA molecule of the expression vector can comprise a sense region and an antisense region. The antisense region can comprise sequence complementary to a RNA or DNA sequence encoding PARK1, PARK2, PARK5, and/or PARK7 and the sense region can comprise sequence complementary to the antisense region. The siNA molecule can comprise two distinct strands having
25 complementary sense and antisense regions. The siNA molecule can comprise a single strand having complementary sense and antisense regions.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against PARK1, PARK2, PARK5, and/or PARK7 inside a cell or reconstituted *in vitro* system,
30 wherein the chemical modification comprises one or more (e.g., about 1, 2, 3, 4, 5, 6, 7,

8, 9, 10, or more) nucleotides comprising a backbone modified internucleotide linkage having Formula I:

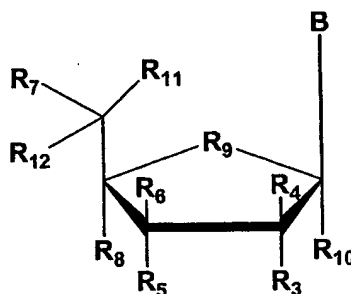


wherein each R1 and R2 is independently any nucleotide, non-nucleotide, or polynucleotide which can be naturally-occurring or chemically-modified, each X and Y is independently O, S, N, alkyl, or substituted alkyl, each Z and W is independently O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, aralkyl, or acetyl and wherein W, X, Y, and Z are optionally not all O. In another embodiment, a backbone modification of the invention comprises a phosphonoacetate and/or thiophosphonoacetate internucleotide linkage (see for example Sheehan et al., 2003, Nucleic Acids Research, 31, 4109-4118).

The chemically-modified internucleotide linkages having Formula I, for example, wherein any Z, W, X, and/or Y independently comprises a sulphur atom, can be present in one or both oligonucleotide strands of the siNA duplex, for example, in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) chemically-modified internucleotide linkages having Formula I at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (e.g., about 1, 2, 3, 4, 5, or more) chemically-modified internucleotide linkages having Formula I at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) pyrimidine nucleotides with chemically-modified internucleotide linkages having Formula I in the sense strand, the antisense strand, or both strands. In yet another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) purine nucleotides with chemically-modified internucleotide linkages having Formula I in the sense strand, the antisense strand, or both strands. In another embodiment, a siNA molecule of the invention having

internucleotide linkage(s) of Formula I also comprises a chemically-modified nucleotide or non-nucleotide having any of Formulae I-VII.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against
 5 PARK1, PARK2, PARK5, and/or PARK7 inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides or non-nucleotides having Formula II:

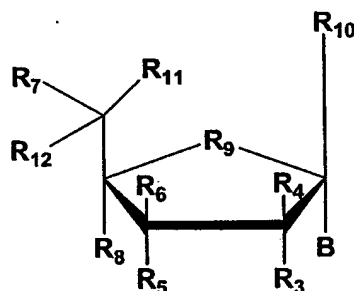


wherein each R3, R4, R5, R6, R7, R8, R10, R11 and R12 is independently H, OH, alkyl,
 10 substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino,
 15 substituted silyl, or group having Formula I or II; R9 is O, S, CH2, S=O, CHF, or CF2, and B is a nucleosidic base such as adenine, guanine, uracil, cytosine, thymine, 2-aminoadenosine, 5-methylcytosine, 2,6-diaminopurine, or any other non-naturally occurring base that can be complementary or non-complementary to target RNA or a non-nucleosidic base such as phenyl, naphthyl, 3-nitropyrrole, 5-nitroindole, nebularine,
 20 pyridone, pyridinone, or any other non-naturally occurring universal base that can be complementary or non-complementary to target RNA.

The chemically-modified nucleotide or non-nucleotide of Formula II can be present in one or both oligonucleotide strands of the siNA duplex, for example in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention
 25 can comprise one or more chemically-modified nucleotide or non-nucleotide of Formula

II at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified nucleotides or non-nucleotides of Formula II at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (*e.g.*, about 1, 2, 3, 4, 5, or more) chemically-modified nucleotides or non-nucleotides of Formula II at the 3'-end of the sense strand, the antisense strand, or both strands.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against PARK1, PARK2, PARK5, and/or PARK7 inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) nucleotides or non-nucleotides having Formula III:



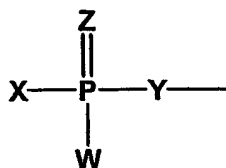
wherein each R3, R4, R5, R6, R7, R8, R10, R11 and R12 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, or group having Formula I or II; R9 is O, S, CH2, S=O, CHF, or CF2, and B is a nucleosidic base such as adenine, guanine, uracil, cytosine, thymine, 2-aminoadenosine, 5-methylcytosine, 2,6-diaminopurine, or any other non-naturally occurring base that can be employed to be complementary or non-complementary to target RNA or a non-nucleosidic base such as phenyl, naphthyl, 3-nitropyrrole, 5-

nitroindole, nebularine, pyridone, pyridinone, or any other non-naturally occurring universal base that can be complementary or non-complementary to target RNA.

The chemically-modified nucleotide or non-nucleotide of Formula III can be present in one or both oligonucleotide strands of the siNA duplex, for example, in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more chemically-modified nucleotide or non-nucleotide of Formula III at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (e.g., about 1, 2, 3, 4, 5, or more) chemically-modified nucleotide(s) or non-nucleotide(s) of Formula III at the 5'-end of the sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (e.g., about 1, 2, 3, 4, 5, or more) chemically-modified nucleotide or non-nucleotide of Formula III at the 3'-end of the sense strand, the antisense strand, or both strands.

In another embodiment, a siNA molecule of the invention comprises a nucleotide having Formula II or III, wherein the nucleotide having Formula II or III is in an inverted configuration. For example, the nucleotide having Formula II or III is connected to the siNA construct in a 3'-3', 3'-2', 2'-3', or 5'-5' configuration, such as at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both siNA strands.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against PARK1, PARK2, PARK5, and/or PARK7 inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises a 5'-terminal phosphate group having Formula IV:



25

wherein each X and Y is independently O, S, N, alkyl, substituted alkyl, or alkylhalo; wherein each Z and W is independently O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, aralkyl, alkylhalo, or acetyl; and wherein W, X, Y and Z are not all O.

In one embodiment, the invention features a siNA molecule having a 5'-terminal phosphate group having Formula IV on the target-complementary strand, for example, a strand complementary to a target RNA, wherein the siNA molecule comprises an all RNA siNA molecule. In another embodiment, the invention features a siNA molecule having a 5'-terminal phosphate group having Formula IV on the target-complementary strand wherein the siNA molecule also comprises about 1 to about 3 (e.g., about 1, 2, or 3) nucleotide 3'-terminal nucleotide overhangs having about 1 to about 4 (e.g., about 1, 2, 3, or 4) deoxyribonucleotides on the 3'-end of one or both strands. In another embodiment, a 5'-terminal phosphate group having Formula IV is present on the target-complementary strand of a siNA molecule of the invention, for example a siNA molecule having chemical modifications having any of Formulae I-VII.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) against PARK1, PARK2, PARK5, and/or PARK7 inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises one or more phosphorothioate internucleotide linkages. For example, in a non-limiting example, the invention features a chemically-modified short interfering nucleic acid (siNA) having about 1, 2, 3, 4, 5, 6, 7, 8 or more phosphorothioate internucleotide linkages in one siNA strand. In yet another embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) individually having about 1, 2, 3, 4, 5, 6, 7, 8 or more phosphorothioate internucleotide linkages in both siNA strands. The phosphorothioate internucleotide linkages can be present in one or both oligonucleotide strands of the siNA duplex, for example in the sense strand, the antisense strand, or both strands. The siNA molecules of the invention can comprise one or more phosphorothioate internucleotide linkages at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand, the antisense strand, or both strands. For example, an exemplary siNA molecule of the invention can comprise about 1 to about 5 or more (e.g., about 1, 2, 3, 4, 5, or more) consecutive phosphorothioate internucleotide linkages at the 5'-end of the

sense strand, the antisense strand, or both strands. In another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) pyrimidine phosphorothioate internucleotide linkages in the sense strand, the antisense strand, or both strands. In yet another non-limiting example, an exemplary siNA molecule of the invention can comprise one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) purine phosphorothioate internucleotide linkages in the sense strand, the antisense strand, or both strands.

In one embodiment, the invention features a siNA molecule, wherein the sense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or about one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 10 or more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In another embodiment, the invention features a siNA molecule, wherein the sense strand comprises about 1 to about 5, specifically about 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense

strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5, or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more, pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without about 1 to about 5 or more, for example about 1, 2, 3, 4, 5, or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In one embodiment, the invention features a siNA molecule, wherein the antisense strand comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more phosphorothioate internucleotide linkages, and/or about one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 10 or more, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3' and 5'-ends, being present in the same or different strand.

In another embodiment, the invention features a siNA molecule, wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more
5 (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the sense strand; and wherein the antisense strand comprises about 1 to about 5 or more, specifically about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) 2'-deoxy, 2'-O-methyl,
10 2'-deoxy-2'-fluoro, and/or one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) universal base modified nucleotides, and optionally a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more pyrimidine nucleotides of the sense and/or antisense siNA strand are chemically-
15 modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without about 1 to about 5, for example about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends, being present in the same or different strand.

In one embodiment, the invention features a chemically-modified short interfering
20 nucleic acid (siNA) molecule having about 1 to about 5, specifically about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages in each strand of the siNA molecule.

In another embodiment, the invention features a siNA molecule comprising 2'-5' internucleotide linkages. The 2'-5' internucleotide linkage(s) can be at the 3'-end, the 5'-end, or both of the 3'- and 5'-ends of one or both siNA sequence strands. In addition, the
25 2'-5' internucleotide linkage(s) can be present at various other positions within one or both siNA sequence strands, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more including every internucleotide linkage of a pyrimidine nucleotide in one or both strands of the siNA molecule can comprise a 2'-5' internucleotide linkage, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more including every internucleotide linkage of a purine nucleotide in
30 one or both strands of the siNA molecule can comprise a 2'-5' internucleotide linkage.

In another embodiment, a chemically-modified siNA molecule of the invention comprises a duplex having two strands, one or both of which can be chemically-modified, wherein each strand is about 18 to about 27 (*e.g.*, about 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27) nucleotides in length, wherein the duplex has about 18 to about 23 (*e.g.*, about 18, 19, 20, 21, 22, or 23) base pairs, and wherein the chemical modification comprises a structure having any of Formulae I-VII. For example, an exemplary chemically-modified siNA molecule of the invention comprises a duplex having two strands, one or both of which can be chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein each strand consists of about 21 nucleotides, each having a 2-nucleotide 3'-terminal nucleotide overhang, and wherein the duplex has about 19 base pairs. In another embodiment, a siNA molecule of the invention comprises a single stranded hairpin structure, wherein the siNA is about 36 to about 70 (*e.g.*, about 36, 40, 45, 50, 55, 60, 65, or 70) nucleotides in length having about 18 to about 23 (*e.g.*, about 18, 19, 20, 21, 22, or 23) base pairs, and wherein the siNA can include a chemical modification comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a linear oligonucleotide having about 42 to about 50 (*e.g.*, about 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides that is chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein the linear oligonucleotide forms a hairpin structure having about 19 base pairs and a 2-nucleotide 3'-terminal nucleotide overhang. In another embodiment, a linear hairpin siNA molecule of the invention contains a stem loop motif, wherein the loop portion of the siNA molecule is biodegradable. For example, a linear hairpin siNA molecule of the invention is designed such that degradation of the loop portion of the siNA molecule *in vivo* can generate a double-stranded siNA molecule with 3'-terminal overhangs, such as 3'-terminal nucleotide overhangs comprising about 2 nucleotides.

In another embodiment, a siNA molecule of the invention comprises a hairpin structure, wherein the siNA is about 25 to about 50 (*e.g.*, about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides in length having about 3 to about 25 (*e.g.*, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) base pairs, and wherein the siNA can include one or

more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a linear oligonucleotide having about 25 to about 35 (*e.g.*, about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35) nucleotides that is chemically-modified with one or more chemical modifications having any of Formulae I-VII or any combination thereof, wherein the linear oligonucleotide forms a hairpin structure having about 3 to about 23 (*e.g.*, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23) base pairs and a 5'-terminal phosphate group that can be chemically modified as described herein (for example a 5'-terminal phosphate group having Formula IV). In another embodiment, a linear hairpin siNA molecule of the invention contains a stem loop motif, wherein the loop portion of the siNA molecule is biodegradable. In one embodiment, a linear hairpin siNA molecule of the invention comprises a loop portion comprising a non-nucleotide linker.

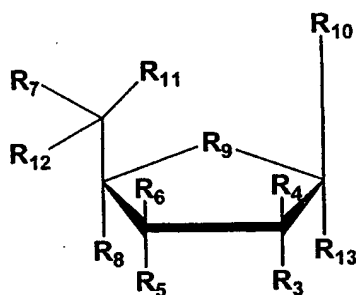
In another embodiment, a siNA molecule of the invention comprises an asymmetric hairpin structure, wherein the siNA is about 25 to about 50 (*e.g.*, about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides in length having about 3 to about 20 (*e.g.*, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) base pairs, and wherein the siNA can include one or more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a linear oligonucleotide having about 25 to about 35 (*e.g.*, about 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35) nucleotides that is chemically-modified with one or more chemical modifications having any of Formulae I-VII or any combination thereof, wherein the linear oligonucleotide forms an asymmetric hairpin structure having about 3 to about 18 (*e.g.*, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18) base pairs and a 5'-terminal phosphate group that can be chemically modified as described herein (for example a 5'-terminal phosphate group having Formula IV). In one embodiment, an asymmetric hairpin siNA molecule of the invention contains a stem loop motif, wherein the loop portion of the siNA molecule is biodegradable. In another embodiment, an asymmetric hairpin siNA molecule of the invention comprises a loop portion comprising a non-nucleotide linker.

In another embodiment, a siNA molecule of the invention comprises an asymmetric double stranded structure having separate polynucleotide strands comprising sense and antisense regions, wherein the antisense region is about 16 to about 25 (*e.g.*, about 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) nucleotides in length, wherein the sense region is about 3 to about 18 (*e.g.*, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18) nucleotides in length, wherein the sense region and the antisense region have at least 3 complementary nucleotides, and wherein the siNA can include one or more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises an asymmetric double stranded structure having separate polynucleotide strands comprising sense and antisense regions, wherein the antisense region is about 18 to about 22 (*e.g.*, about 18, 19, 20, 21, or 22) nucleotides in length and wherein the sense region is about 3 to about 15 (*e.g.*, about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) nucleotides in length, wherein the sense region the antisense region have at least 3 complementary nucleotides, and wherein the siNA can include one or more chemical modifications comprising a structure having any of Formulae I-VII or any combination thereof. In another embodiment, the asymmetric double stranded siNA molecule can also have a 5'-terminal phosphate group that can be chemically modified as described herein (for example a 5'-terminal phosphate group having Formula IV).

In another embodiment, a siNA molecule of the invention comprises a circular nucleic acid molecule, wherein the siNA is about 38 to about 70 (*e.g.*, about 38, 40, 45, 50, 55, 60, 65, or 70) nucleotides in length having about 18 to about 23 (*e.g.*, about 18, 19, 20, 21, 22, or 23) base pairs, and wherein the siNA can include a chemical modification, which comprises a structure having any of Formulae I-VII or any combination thereof. For example, an exemplary chemically-modified siNA molecule of the invention comprises a circular oligonucleotide having about 42 to about 50 (*e.g.*, about 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotides that is chemically-modified with a chemical modification having any of Formulae I-VII or any combination thereof, wherein the circular oligonucleotide forms a dumbbell shaped structure having about 19 base pairs and 2 loops.

In another embodiment, a circular siNA molecule of the invention contains two loop motifs, wherein one or both loop portions of the siNA molecule is biodegradable. For example, a circular siNA molecule of the invention is designed such that degradation of the loop portions of the siNA molecule *in vivo* can generate a double-stranded siNA molecule with 3'-terminal overhangs, such as 3'-terminal nucleotide overhangs comprising about 2 nucleotides.

In one embodiment, a siNA molecule of the invention comprises at least one (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) abasic moiety, for example a compound having Formula V:



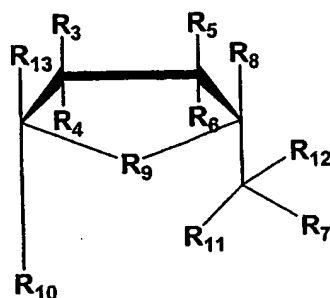
10

wherein each R3, R4, R5, R6, R7, R8, R10, R11, R12, and R13 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, or group having Formula I or II; R9 is O, S, CH2, S=O, CHF, or CF2.

15

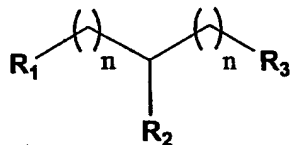
In one embodiment, a siNA molecule of the invention comprises at least one (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) inverted abasic moiety, for example a compound having Formula VI:

20



wherein each R3, R4, R5, R6, R7, R8, R10, R11, R12, and R13 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, or group having Formula I or II; R9 is O, S, CH2, S=O, CHF, or CF2, and either R2, R3, R8 or R13 serve as points of attachment to the siNA molecule of the invention.

In another embodiment, a siNA molecule of the invention comprises at least one (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) substituted polyalkyl moieties, for example a compound having Formula VII:



wherein each n is independently an integer from 1 to 12, each R1, R2 and R3 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, or a group having Formula I, and R1, R2 or R3 serves as points of attachment to the siNA molecule of the invention.

In another embodiment, the invention features a compound having Formula VII, wherein R1 and R2 are hydroxyl (OH) groups, $n = 1$, and R3 comprises O and is the point of attachment to the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both strands of a double-stranded siNA molecule of the invention or to a single-stranded siNA molecule of the invention. This modification is referred to herein as "glyceryl" (for example modification 6 in Figure 10).

In another embodiment, a moiety having any of Formula V, VI or VII of the invention is at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of a siNA molecule of the invention. For example, a moiety having Formula V, VI or VII can be present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense strand, the sense strand, or both antisense and sense strands of the siNA molecule. In addition, a moiety having Formula VII can be present at the 3'-end or the 5'-end of a hairpin siNA molecule as described herein.

In another embodiment, a siNA molecule of the invention comprises an abasic residue having Formula V or VI, wherein the abasic residue having Formula VI or VI is connected to the siNA construct in a 3'-3', 3'-2', 2'-3', or 5'-5' configuration, such as at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of one or both siNA strands.

In one embodiment, a siNA molecule of the invention comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) locked nucleic acid (LNA) nucleotides, for example at the 5'-end, the 3'-end, both of the 5' and 3'-ends, or any combination thereof, of the siNA molecule.

In another embodiment, a siNA molecule of the invention comprises one or more (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) acyclic nucleotides, for example at the 5'-end, the 3'-end, both of the 5' and 3'-ends, or any combination thereof, of the siNA molecule.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-

2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality
5 of purine nucleotides are 2'-deoxy purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the sense region are 2'-deoxy purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides), wherein any nucleotides
10 comprising a 3'-terminal nucleotide overhang that are present in said sense region are 2'-deoxy nucleotides.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the sense region are 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides).
20
25

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising a sense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), wherein any (*e.g.*, one or more or all) purine
30

nucleotides present in the sense region are 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said sense region are 2'-deoxy nucleotides.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), wherein any (*e.g.*, one or more or all) purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (*e.g.*, wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and wherein any nucleotides comprising a 3'-terminal nucleotide overhang that are present in said antisense region are 2'-deoxy nucleotides.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein any (*e.g.*, one or more or all) pyrimidine nucleotides present in the antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (*e.g.*, wherein all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (*e.g.*, one or

more or all) purine nucleotides present in the antisense region are 2'-deoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering
5 nucleic acid (siNA) molecule of the invention comprising an antisense region, wherein
any (e.g., one or more or all) pyrimidine nucleotides present in the antisense region are
2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are
2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine
nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any (e.g., one or
10 more or all) purine nucleotides present in the antisense region are 2'-O-methyl purine
nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or
alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides).

In one embodiment, the invention features a chemically-modified short interfering
nucleic acid (siNA) molecule of the invention capable of mediating RNA interference
15 (RNAi) against PARK1, PARK2, PARK5, and/or PARK7 inside a cell or reconstituted
in vitro system comprising a sense region, wherein one or more pyrimidine nucleotides
present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein
all pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a
plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and
20 one or more purine nucleotides present in the sense region are 2'-deoxy purine
nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or
alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides), and an
antisense region, wherein one or more pyrimidine nucleotides present in the antisense
region are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine
25 nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of
pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and one or more
purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides
(e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a
plurality of purine nucleotides are 2'-O-methyl purine nucleotides). The sense region
30 and/or the antisense region can have a terminal cap modification, such as any
modification described herein or shown in Figure 10, that is optionally present at the 3'-

end, the 5'-end, or both of the 3' and 5'-ends of the sense and/or antisense sequence. The sense and/or antisense region can optionally further comprise a 3'-terminal nucleotide overhang having about 1 to about 4 (e.g., about 1, 2, 3, or 4) 2'-deoxynucleotides. The overhang nucleotides can further comprise one or more (e.g., about 1, 2, 3, 4 or more) phosphorothioate, phosphonoacetate, and/or thiophosphonoacetate internucleotide linkages. Non-limiting examples of these chemically-modified siNAs are shown in **Figures 4 and 5 and Tables III and IV** herein. In any of these described embodiments, the purine nucleotides present in the sense region are alternatively 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides) and one or more purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides). Also, in any of these embodiments, one or more purine nucleotides present in the sense region are alternatively purine ribonucleotides (e.g., wherein all purine nucleotides are purine ribonucleotides or alternately a plurality of purine nucleotides are purine ribonucleotides) and any purine nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides). Additionally, in any of these embodiments, one or more purine nucleotides present in the sense region and/or present in the antisense region are alternatively selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides (e.g., wherein all purine nucleotides are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides or alternately a plurality of purine nucleotides are selected from the group consisting of 2'-deoxy nucleotides, locked nucleic acid (LNA) nucleotides, 2'-methoxyethyl nucleotides, 4'-thionucleotides, and 2'-O-methyl nucleotides).

In another embodiment, any modified nucleotides present in the siNA molecules of the invention, preferably in the antisense strand of the siNA molecules of the invention, but also optionally in the sense and/or both antisense and sense strands, comprise

modified nucleotides having properties or characteristics similar to naturally occurring ribonucleotides. For example, the invention features siNA molecules including modified nucleotides having a Northern conformation (e.g., Northern pseudorotation cycle, see for example Saenger, *Principles of Nucleic Acid Structure*, Springer-Verlag ed., 1984). As
5 such, chemically modified nucleotides present in the siNA molecules of the invention, preferably in the antisense strand of the siNA molecules of the invention, but also optionally in the sense and/or both antisense and sense strands, are resistant to nuclease degradation while at the same time maintaining the capacity to mediate RNAi. Non-limiting examples of nucleotides having a northern configuration include locked nucleic
10 acid (LNA) nucleotides (e.g., 2'-O, 4'-C-methylene-(D-ribofuranosyl) nucleotides); 2'-methoxyethoxy (MOE) nucleotides; 2'-methyl-thio-ethyl, 2'-deoxy-2'-fluoro nucleotides, 2'-deoxy-2'-chloro nucleotides, 2'-azido nucleotides, and 2'-O-methyl nucleotides.

In one embodiment, the sense strand of a double stranded siNA molecule of the
15 invention comprises a terminal cap moiety, (see for example Figure 10) such as an inverted deoxyabaisc moiety, at the 3'-end, 5'-end, or both 3' and 5'-ends of the sense strand.

In one embodiment, the invention features a chemically-modified short interfering nucleic acid molecule (siNA) capable of mediating RNA interference (RNAi) against
20 PARK1, PARK2, PARK5, and/or PARK7 inside a cell or reconstituted *in vitro* system, wherein the chemical modification comprises a conjugate covalently attached to the chemically-modified siNA molecule. Non-limiting examples of conjugates contemplated by the invention include conjugates and ligands described in Vargeese *et al.*, USSN 10/427,160, filed April 30, 2003, incorporated by reference herein in its
25 entirety, including the drawings. In another embodiment, the conjugate is covalently attached to the chemically-modified siNA molecule via a biodegradable linker. In one embodiment, the conjugate molecule is attached at the 3'-end of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule. In another embodiment, the conjugate molecule is attached at the 5'-end of either the sense
30 strand, the antisense strand, or both strands of the chemically-modified siNA molecule. In yet another embodiment, the conjugate molecule is attached both the 3'-end and 5'-end

of either the sense strand, the antisense strand, or both strands of the chemically-modified siNA molecule, or any combination thereof. In one embodiment, a conjugate molecule of the invention comprises a molecule that facilitates delivery of a chemically-modified siNA molecule into a biological system, such as a cell. In another embodiment, the conjugate molecule attached to the chemically-modified siNA molecule is a polyethylene glycol, human serum albumin, or a ligand for a cellular receptor that can mediate cellular uptake. Examples of specific conjugate molecules contemplated by the instant invention that can be attached to chemically-modified siNA molecules are described in Vargeese *et al.*, U.S. Serial No. 10/201,394, filed July 22, 2002 incorporated by reference herein. The type of conjugates used and the extent of conjugation of siNA molecules of the invention can be evaluated for improved pharmacokinetic profiles, bioavailability, and/or stability of siNA constructs while at the same time maintaining the ability of the siNA to mediate RNAi activity. As such, one skilled in the art can screen siNA constructs that are modified with various conjugates to determine whether the siNA conjugate complex possesses improved properties while maintaining the ability to mediate RNAi, for example in animal models as are generally known in the art.

In one embodiment, the invention features a short interfering nucleic acid (siNA) molecule of the invention, wherein the siNA further comprises a nucleotide, non-nucleotide, or mixed nucleotide/non-nucleotide linker that joins the sense region of the siNA to the antisense region of the siNA. In one embodiment, a nucleotide linker of the invention can be a linker of ≥ 2 nucleotides in length, for example about 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in length. In another embodiment, the nucleotide linker can be a nucleic acid aptamer. By "aptamer" or "nucleic acid aptamer" as used herein is meant a nucleic acid molecule that binds specifically to a target molecule wherein the nucleic acid molecule has sequence that comprises a sequence recognized by the target molecule in its natural setting. Alternately, an aptamer can be a nucleic acid molecule that binds to a target molecule where the target molecule does not naturally bind to a nucleic acid. The target molecule can be any molecule of interest. For example, the aptamer can be used to bind to a ligand-binding domain of a protein, thereby preventing interaction of the naturally occurring ligand with the protein. This is a non-limiting example and those in the art will recognize that other embodiments can be readily generated using techniques generally known in the art. (See, for example, Gold *et al.*, 1995, *Annu. Rev.*

Biochem., 64, 763; Brody and Gold, 2000, *J. Biotechnol.*, 74, 5; Sun, 2000, *Curr. Opin. Mol. Ther.*, 2, 100; Kusser, 2000, *J. Biotechnol.*, 74, 27; Hermann and Patel, 2000, *Science*, 287, 820; and Jayasena, 1999, *Clinical Chemistry*, 45, 1628.)

In yet another embodiment, a non-nucleotide linker of the invention comprises
5 abasic nucleotide, polyether, polyamine, polyamide, peptide, carbohydrate, lipid, polyhydrocarbon, or other polymeric compounds (e.g. polyethylene glycols such as those having between 2 and 100 ethylene glycol units). Specific examples include those described by Seela and Kaiser, *Nucleic Acids Res.* 1990, 18:6353 and *Nucleic Acids Res.* 1987, 15:3113; Cload and Schepartz, *J. Am. Chem. Soc.* 1991, 113:6324; Richardson and
10 Schepartz, *J. Am. Chem. Soc.* 1991, 113:5109; Ma *et al.*, *Nucleic Acids Res.* 1993, 21:2585 and *Biochemistry* 1993, 32:1751; Durand *et al.*, *Nucleic Acids Res.* 1990, 18:6353; McCurdy *et al.*, *Nucleosides & Nucleotides* 1991, 10:287; Jschke *et al.*, *Tetrahedron Lett.* 1993, 34:301; Ono *et al.*, *Biochemistry* 1991, 30:9914; Arnold *et al.*, International Publication No. WO 89/02439; Usman *et al.*, International Publication No.
15 WO 95/06731; Dudycz *et al.*, International Publication No. WO 95/11910 and Ferentz and Verdine, *J. Am. Chem. Soc.* 1991, 113:4000, all hereby incorporated by reference herein. A "non-nucleotide" further means any group or compound that can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to
20 exhibit their enzymatic activity. The group or compound can be abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine, for example at the C1 position of the sugar.

In one embodiment, the invention features a short interfering nucleic acid (siNA) molecule capable of mediating RNA interference (RNAi) inside a cell or reconstituted *in*
25 *vitro* system, wherein one or both strands of the siNA molecule that are assembled from two separate oligonucleotides do not comprise any ribonucleotides. For example, a siNA molecule can be assembled from a single oligonucleotide where the sense and antisense regions of the siNA comprise separate oligonucleotides that do not have any ribonucleotides (e.g., nucleotides having a 2'-OH group) present in the oligonucleotides.
30 In another example, a siNA molecule can be assembled from a single oligonucleotide where the sense and antisense regions of the siNA are linked or circularized by a

nucleotide or non-nucleotide linker as described herein, wherein the oligonucleotide does not have any ribonucleotides (e.g., nucleotides having a 2'-OH group) present in the oligonucleotide. Applicant has surprisingly found that the presence of ribonucleotides (e.g., nucleotides having a 2'-hydroxyl group) within the siNA molecule is not required or essential to support RNAi activity. As such, in one embodiment, all positions within the siNA can include chemically modified nucleotides and/or non-nucleotides such as nucleotides and or non-nucleotides having Formula I, II, III, IV, V, VI, or VII or any combination thereof to the extent that the ability of the siNA molecule to support RNAi activity in a cell is maintained.

10 In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted *in vitro* system comprising a single stranded polynucleotide having complementarity to a target nucleic acid sequence. In another embodiment, the single stranded siNA molecule of the invention comprises a 5'-terminal phosphate group. In another embodiment, the single
15 stranded siNA molecule of the invention comprises a 5'-terminal phosphate group and a 3'-terminal phosphate group (e.g., a 2',3'-cyclic phosphate). In another embodiment, the single stranded siNA molecule of the invention comprises about 19 to about 29 (e.g., about 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) nucleotides. In yet another embodiment, the single stranded siNA molecule of the invention comprises one or more
20 chemically modified nucleotides or non-nucleotides described herein. For example, all the positions within the siNA molecule can include chemically-modified nucleotides such as nucleotides having any of Formulae I-VII, or any combination thereof to the extent that the ability of the siNA molecule to support RNAi activity in a cell is maintained.

25 In one embodiment, a siNA molecule of the invention is a single stranded siNA molecule that mediates RNAi activity in a cell or reconstituted *in vitro* system comprising a single stranded polynucleotide having complementarity to a target nucleic acid sequence, wherein one or more pyrimidine nucleotides present in the siNA are 2'-deoxy-2'-fluoro pyrimidine nucleotides (e.g., wherein all pyrimidine nucleotides are 2'-
30 deoxy-2'-fluoro pyrimidine nucleotides or alternately a plurality of pyrimidine nucleotides are 2'-deoxy-2'-fluoro pyrimidine nucleotides), and wherein any purine

nucleotides present in the antisense region are 2'-O-methyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-O-methyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-O-methyl purine nucleotides), and a terminal cap modification, such as any modification described herein or shown in Figure 10, that is optionally present at the 3'-end, the 5'-end, or both of the 3' and 5'-ends of the antisense sequence. The siNA optionally further comprises about 1 to about 4 or more (e.g., about 1, 2, 3, 4 or more) terminal 2'-deoxynucleotides at the 3'-end of the siNA molecule, wherein the terminal nucleotides can further comprise one or more (e.g., 1, 2, 3, 4 or more) phosphorothioate, phosphonoacetate, and/or thiophosphonoacetate internucleotide linkages, and wherein the siNA optionally further comprises a terminal phosphate group, such as a 5'-terminal phosphate group. In any of these embodiments, any purine nucleotides present in the antisense region are alternatively 2'-deoxy purine nucleotides (e.g., wherein all purine nucleotides are 2'-deoxy purine nucleotides or alternately a plurality of purine nucleotides are 2'-deoxy purine nucleotides). Also, in any of these embodiments, any purine nucleotides present in the siNA (i.e., purine nucleotides present in the sense and/or antisense region) can alternatively be locked nucleic acid (LNA) nucleotides (e.g., wherein all purine nucleotides are LNA nucleotides or alternately a plurality of purine nucleotides are LNA nucleotides). Also, in any of these embodiments, any purine nucleotides present in the siNA are alternatively 2'-methoxyethyl purine nucleotides (e.g., wherein all purine nucleotides are 2'-methoxyethyl purine nucleotides or alternately a plurality of purine nucleotides are 2'-methoxyethyl purine nucleotides). In another embodiment, any modified nucleotides present in the single stranded siNA molecules of the invention comprise modified nucleotides having properties or characteristics similar to naturally occurring ribonucleotides. For example, the invention features siNA molecules including modified nucleotides having a Northern conformation (e.g., Northern pseudorotation cycle, see for example Saenger, *Principles of Nucleic Acid Structure*, Springer-Verlag ed., 1984). As such, chemically modified nucleotides present in the single stranded siNA molecules of the invention are preferably resistant to nuclease degradation while at the same time maintaining the capacity to mediate RNAi.

In one embodiment, the invention features a method for modulating the expression of a PARK1, PARK2, PARK5, and/or PARK7 gene within a cell comprising: (a)

synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the PARK1, PARK2, PARK5, and/or PARK7 gene; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the PARK1, PARK2, PARK5, and/or PARK7 gene in the cell.

In one embodiment, the invention features a method for modulating the expression of a PARK1, PARK2, PARK5, and/or PARK7 gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the PARK1, PARK2, PARK5, and/or PARK7 gene and wherein the sense strand sequence of the siNA comprises a sequence identical or substantially similar to the sequence of the target RNA; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the PARK1, PARK2, PARK5, and/or PARK7 gene in the cell.

In another embodiment, the invention features a method for modulating the expression of more than one PARK1, PARK2, PARK5, and/or PARK7 gene within a cell comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the PARK1, PARK2, PARK5, and/or PARK7 genes; and (b) introducing the siNA molecules into a cell under conditions suitable to modulate the expression of the PARK1, PARK2, PARK5, and/or PARK7 genes in the cell.

In another embodiment, the invention features a method for modulating the expression of two or more PARK1, PARK2, PARK5, and/or PARK7 genes within a cell comprising: (a) synthesizing one or more siNA molecules of the invention, which can be chemically-modified, wherein the siNA strands comprise sequences complementary to RNA of the PARK1, PARK2, PARK5, and/or PARK7 genes and wherein the sense strand sequences of the siNAs comprise sequences identical or substantially similar to the sequences of the target RNAs; and (b) introducing the siNA molecules into a cell under conditions suitable to modulate the expression of the PARK1, PARK2, PARK5, and/or PARK7 genes in the cell.

In another embodiment, the invention features a method for modulating the expression of more than one PARK1, PARK2, PARK5, and/or PARK7 gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence
5 complementary to RNA of the PARK1, PARK2, PARK5, and/or PARK7 gene and wherein the sense strand sequence of the siNA comprises a sequence identical or substantially similar to the sequences of the target RNAs; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the PARK1, PARK2, PARK5, and/or PARK7 genes in the cell.

10 In one embodiment, siNA molecules of the invention are used as reagents in *ex vivo* applications. For example, siNA reagents are introduced into tissue or cells that are transplanted into a subject for therapeutic effect. The cells and/or tissue can be derived from an organism or subject that later receives the explant, or can be derived from another organism or subject prior to transplantation. The siNA molecules can be used to
15 modulate the expression of one or more genes in the cells or tissue, such that the cells or tissue obtain a desired phenotype or are able to perform a function when transplanted in vivo. In one embodiment, certain target cells from a patient are extracted. These extracted cells are contacted with siNAs targeting a specific nucleotide sequence within the cells under conditions suitable for uptake of the siNAs by these cells (e.g. using
20 delivery reagents such as cationic lipids, liposomes and the like or using techniques such as electroporation to facilitate the delivery of siNAs into cells). The cells are then reintroduced back into the same patient or other patients. In one embodiment, the invention features a method of modulating the expression of a PARK1, PARK2, PARK5, and/or PARK7 gene in a tissue explant comprising: (a) synthesizing a siNA molecule of
25 the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the PARK1, PARK2, PARK5, and/or PARK7 gene; and (b) introducing the siNA molecule into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the PARK1, PARK2, PARK5, and/or PARK7 gene in the tissue explant. In another
30 embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable

to modulate the expression of the PARK1, PARK2, PARK5, and/or PARK7 gene in that organism.

In one embodiment, the invention features a method of modulating the expression of a PARK1, PARK2, PARK5, and/or PARK7 gene in a tissue explant comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the PARK1, PARK2, PARK5, and/or PARK7 gene and wherein the sense strand sequence of the siNA comprises a sequence identical or substantially similar to the sequence of the target RNA; and (b) introducing the siNA molecule into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the PARK1, PARK2, PARK5, and/or PARK7 gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the PARK1, PARK2, PARK5, and/or PARK7 gene in that organism.

In another embodiment, the invention features a method of modulating the expression of more than one PARK1, PARK2, PARK5, and/or PARK7 gene in a tissue explant comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the PARK1, PARK2, PARK5, and/or PARK7 genes; and (b) introducing the siNA molecules into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the PARK1, PARK2, PARK5, and/or PARK7 genes in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the PARK1, PARK2, PARK5, and/or PARK7 genes in that organism.

In one embodiment, the invention features a method of modulating the expression of a PARK1, PARK2, PARK5, and/or PARK7 gene in an organism comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the PARK1, PARK2, PARK5, and/or PARK7 gene; and (b) introducing the siNA molecule

into the organism under conditions suitable to modulate the expression of the PARK1, PARK2, PARK5, and/or PARK7 gene in the organism. The level of PARK1, PARK2, PARK5, and/or PARK7 protein or RNA can be determined as is known in the art.

In another embodiment, the invention features a method of modulating the expression of more than one PARK1, PARK2, PARK5, and/or PARK7 gene in an organism comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein one of the siNA strands comprises a sequence complementary to RNA of the PARK1, PARK2, PARK5, and/or PARK7 genes; and (b) introducing the siNA molecules into the organism under conditions suitable to modulate the expression of the PARK1, PARK2, PARK5, and/or PARK7 genes in the organism. The level of PARK1, PARK2, PARK5, and/or PARK7 protein or RNA can be determined as is known in the art.

In one embodiment, the invention features a method for modulating the expression of a PARK1, PARK2, PARK5, and/or PARK7 gene within a cell comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the PARK1, PARK2, PARK5, and/or PARK7 gene; and (b) introducing the siNA molecule into a cell under conditions suitable to modulate the expression of the PARK1, PARK2, PARK5, and/or PARK7 gene in the cell.

In another embodiment, the invention features a method for modulating the expression of more than one PARK1, PARK2, PARK5, and/or PARK7 gene within a cell comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the PARK1, PARK2, PARK5, and/or PARK7 gene; and (b) contacting the cell *in vitro* or *in vivo* with the siNA molecule under conditions suitable to modulate the expression of the PARK1, PARK2, PARK5, and/or PARK7 genes in the cell.

In one embodiment, the invention features a method of modulating the expression of a PARK1, PARK2, PARK5, and/or PARK7 gene in a tissue explant comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified,

wherein the siNA comprises a single stranded sequence having complementarity to RNA of the PARK1, PARK2, PARK5, and/or PARK7 gene; and (b) contacting the cell of the tissue explant derived from a particular organism with the siNA molecule under conditions suitable to modulate the expression of the PARK1, PARK2, PARK5, and/or
5 PARK7 gene in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the PARK1, PARK2, PARK5, and/or PARK7 gene in that organism.

In another embodiment, the invention features a method of modulating the
10 expression of more than one PARK1, PARK2, PARK5, and/or PARK7 gene in a tissue explant comprising: (a) synthesizing siNA molecules of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the PARK1, PARK2, PARK5, and/or PARK7 gene; and (b)
15 introducing the siNA molecules into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the PARK1, PARK2, PARK5, and/or PARK7 genes in the tissue explant. In another embodiment, the method further comprises introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the PARK1, PARK2, PARK5, and/or PARK7 genes in that organism.

In one embodiment, the invention features a method of modulating the expression
20 of a PARK1, PARK2, PARK5, and/or PARK7 gene in an organism comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the PARK1, PARK2, PARK5, and/or PARK7 gene; and (b) introducing the siNA
25 molecule into the organism under conditions suitable to modulate the expression of the PARK1, PARK2, PARK5, and/or PARK7 gene in the organism.

In another embodiment, the invention features a method of modulating the expression of more than one PARK1, PARK2, PARK5, and/or PARK7 gene in an organism comprising: (a) synthesizing siNA molecules of the invention, which can be
30 chemically-modified, wherein the siNA comprises a single stranded sequence having complementarity to RNA of the PARK1, PARK2, PARK5, and/or PARK7 gene; and (b)

introducing the siNA molecules into the organism under conditions suitable to modulate the expression of the PARK1, PARK2, PARK5, and/or PARK7 genes in the organism.

In one embodiment, the invention features a method of modulating the expression of a PARK1, PARK2, PARK5, and/or PARK7 gene in an organism comprising
5 contacting the organism with a siNA molecule of the invention under conditions suitable to modulate the expression of the PARK1, PARK2, PARK5, and/or PARK7 gene in the organism.

In one embodiment, the invention features a method for treating or preventing Parkinson's disease in an organism comprising contacting the organism with a siNA
10 molecule of the invention under conditions suitable to modulate the expression of the PARK1, PARK2, PARK5, and/or PARK7 gene in the organism.

In one embodiment, the invention features a method for treating or preventing Alzheimer's disease in an organism comprising contacting the organism with a siNA molecule of the invention under conditions suitable to modulate the expression of the
15 PARK1, PARK2, PARK5, and/or PARK7 gene in the organism.

In one embodiment, the invention features a method for treating or preventing dementia in an organism comprising contacting the organism with a siNA molecule of the invention under conditions suitable to modulate the expression of the PARK1, PARK2, PARK5, and/or PARK7 gene in the organism.

20 In another embodiment, the invention features a method of modulating the expression of more than one PARK1, PARK2, PARK5, and/or PARK7 genes in an organism comprising contacting the organism with one or more siNA molecules of the invention under conditions suitable to modulate the expression of the PARK1, PARK2, PARK5, and/or PARK7 genes in the organism.

25 The siNA molecules of the invention can be designed to down regulate or inhibit target (e.g., PARK1, PARK2, PARK5, and/or PARK7) gene expression through RNAi targeting of a variety of RNA molecules. In one embodiment, the siNA molecules of the invention are used to target various RNAs corresponding to a target gene. Non-limiting examples of such RNAs include messenger RNA (mRNA), alternate RNA splice

variants of target gene(s), post-transcriptionally modified RNA of target gene(s), pre-mRNA of target gene(s), and/or RNA templates. If alternate splicing produces a family of transcripts that are distinguished by usage of appropriate exons, the instant invention can be used to inhibit gene expression through the appropriate exons to specifically
5 inhibit or to distinguish among the functions of gene family members. For example, a protein that contains an alternatively spliced transmembrane domain can be expressed in both membrane bound and secreted forms. Use of the invention to target the exon containing the transmembrane domain can be used to determine the functional consequences of pharmaceutical targeting of membrane bound as opposed to the secreted
10 form of the protein. Non-limiting examples of applications of the invention relating to targeting these RNA molecules include therapeutic pharmaceutical applications, pharmaceutical discovery applications, molecular diagnostic and gene function applications, and gene mapping, for example using single nucleotide polymorphism mapping with siNA molecules of the invention. Such applications can be implemented
15 using known gene sequences or from partial sequences available from an expressed sequence tag (EST).

In another embodiment, the siNA molecules of the invention are used to target conserved sequences corresponding to a gene family or gene families such as PARK1, PARK2, PARK5, and/or PARK7 family genes. As such, siNA molecules targeting
20 multiple PARK1, PARK2, PARK5, and/or PARK7 targets can provide increased therapeutic effect. In addition, siNA can be used to characterize pathways of gene function in a variety of applications. For example, the present invention can be used to inhibit the activity of target gene(s) in a pathway to determine the function of uncharacterized gene(s) in gene function analysis, mRNA function analysis, or
25 translational analysis. The invention can be used to determine potential target gene pathways involved in various diseases and conditions toward pharmaceutical development. The invention can be used to understand pathways of gene expression involved in, for example, a neurodegenerative disease such as Parkinson's disease, Alzheimer's disease, and/or dementia.

30 In one embodiment, siNA molecule(s) and/or methods of the invention are used to down regulate the expression of gene(s) that encode RNA referred to by Genbank

Accession numbers, for example PARK1, PARK2, PARK5, and/or PARK7 genes encoding RNA sequence(s) referred to herein by Genbank Accession number, for example, Genbank Accession Nos. shown in Table I.

In one embodiment, the invention features a method comprising: (a) generating a
5 library of siNA constructs having a predetermined complexity; and (b) assaying the siNA
constructs of (a) above, under conditions suitable to determine RNAi target sites within
the target RNA sequence. In one embodiment, the siNA molecules of (a) have strands of
a fixed length, for example, about 23 nucleotides in length. In another embodiment, the
siNA molecules of (a) are of differing length, for example having strands of about 19 to
10 about 25 (e.g., about 19, 20, 21, 22, 23, 24, or 25) nucleotides in length. In one
embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described
herein. In another embodiment, the assay can comprise a cell culture system in which
target RNA is expressed. In another embodiment, fragments of target RNA are analyzed
for detectable levels of cleavage, for example by gel electrophoresis, northern blot
15 analysis, or RNase protection assays, to determine the most suitable target site(s) within
the target RNA sequence. The target RNA sequence can be obtained as is known in the
art, for example, by cloning and/or transcription for *in vitro* systems, and by cellular
expression in *in vivo* systems.

In one embodiment, the invention features a method comprising: (a) generating a
20 randomized library of siNA constructs having a predetermined complexity, such as of 4^N ,
where N represents the number of base paired nucleotides in each of the siNA construct
strands (eg. for a siNA construct having 21 nucleotide sense and antisense strands with
19 base pairs, the complexity would be 4^{19}); and (b) assaying the siNA constructs of (a)
above, under conditions suitable to determine RNAi target sites within the target
25 PARK1, PARK2, PARK5, and/or PARK7 RNA sequence. In another embodiment, the
siNA molecules of (a) have strands of a fixed length, for example about 23 nucleotides in
length. In yet another embodiment, the siNA molecules of (a) are of differing length, for
example having strands of about 19 to about 25 (e.g., about 19, 20, 21, 22, 23, 24, or 25)
nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in*
30 *vitro* siNA assay as described in Example 7 herein. In another embodiment, the assay
can comprise a cell culture system in which target RNA is expressed. In another

embodiment, fragments of PARK1, PARK2, PARK5, and/or PARK7 RNA are analyzed for detectable levels of cleavage, for example by gel electrophoresis, northern blot analysis, or RNase protection assays, to determine the most suitable target site(s) within the target PARK1, PARK2, PARK5, and/or PARK7 RNA sequence. The target PARK1, PARK2, PARK5, and/or PARK7 RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for *in vitro* systems, and by cellular expression in *in vivo* systems.

In another embodiment, the invention features a method comprising: (a) analyzing the sequence of a RNA target encoded by a target gene; (b) synthesizing one or more sets of siNA molecules having sequence complementary to one or more regions of the RNA of (a); and (c) assaying the siNA molecules of (b) under conditions suitable to determine RNAi targets within the target RNA sequence. In one embodiment, the siNA molecules of (b) have strands of a fixed length, for example about 23 nucleotides in length. In another embodiment, the siNA molecules of (b) are of differing length, for example having strands of about 19 to about 25 (e.g., about 19, 20, 21, 22, 23, 24, or 25) nucleotides in length. In one embodiment, the assay can comprise a reconstituted *in vitro* siNA assay as described herein. In another embodiment, the assay can comprise a cell culture system in which target RNA is expressed. Fragments of target RNA are analyzed for detectable levels of cleavage, for example by gel electrophoresis, northern blot analysis, or RNase protection assays, to determine the most suitable target site(s) within the target RNA sequence. The target RNA sequence can be obtained as is known in the art, for example, by cloning and/or transcription for *in vitro* systems, and by expression in *in vivo* systems.

By "target site" is meant a sequence within a target RNA that is "targeted" for cleavage mediated by a siNA construct which contains sequences within its antisense region that are complementary to the target sequence.

By "detectable level of cleavage" is meant cleavage of target RNA (and formation of cleaved product RNAs) to an extent sufficient to discern cleavage products above the background of RNAs produced by random degradation of the target RNA. Production of cleavage products from 1-5% of the target RNA is sufficient to detect above the background for most methods of detection.

In one embodiment, the invention features a composition comprising a siNA molecule of the invention, which can be chemically-modified, in a pharmaceutically acceptable carrier or diluent. In another embodiment, the invention features a pharmaceutical composition comprising siNA molecules of the invention, which can be chemically-modified, targeting one or more genes in a pharmaceutically acceptable carrier or diluent. In another embodiment, the invention features a method for diagnosing a disease or condition in a subject comprising administering to the subject a composition of the invention under conditions suitable for the diagnosis of the disease or condition in the subject. In another embodiment, the invention features a method for treating or preventing a disease or condition in a subject, comprising administering to the subject a composition of the invention under conditions suitable for the treatment or prevention of the disease or condition in the subject, alone or in conjunction with one or more other therapeutic compounds. In yet another embodiment, the invention features a method for reducing or preventing a neurologic disease (e.g., Parkinson's disease, Alzheimer's disease, and/or dementia) in a subject, comprising administering to the subject a composition of the invention under conditions suitable for the reduction or prevention of the neurologic disease in the subject.

In another embodiment, the invention features a method for validating a PARK1, PARK2, PARK5, and/or PARK7 gene target, comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands includes a sequence complementary to RNA of a PARK1, PARK2, PARK5, and/or PARK7 target gene; (b) introducing the siNA molecule into a cell, tissue, or organism under conditions suitable for modulating expression of the PARK1, PARK2, PARK5, and/or PARK7 target gene in the cell, tissue, or organism; and (c) determining the function of the gene by assaying for any phenotypic change in the cell, tissue, or organism.

In another embodiment, the invention features a method for validating a PARK1, PARK2, PARK5, and/or PARK7 target comprising: (a) synthesizing a siNA molecule of the invention, which can be chemically-modified, wherein one of the siNA strands includes a sequence complementary to RNA of a PARK1, PARK2, PARK5, and/or PARK7 target gene; (b) introducing the siNA molecule into a biological system under

conditions suitable for modulating expression of the PARK1, PARK2, PARK5, and/or PARK7 target gene in the biological system; and (c) determining the function of the gene by assaying for any phenotypic change in the biological system.

By "biological system" is meant, material, in a purified or unpurified form, from
5 biological sources, including but not limited to human or animal, wherein the system comprises the components required for RNAi activity. The term "biological system" includes, for example, a cell, tissue, or organism, or extract thereof. The term biological system also includes reconstituted RNAi systems that can be used in an *in vitro* setting.

By "phenotypic change" is meant any detectable change to a cell that occurs in
10 response to contact or treatment with a nucleic acid molecule of the invention (e.g., siNA). Such detectable changes include, but are not limited to, changes in shape, size, proliferation, motility, protein expression or RNA expression or other physical or chemical changes as can be assayed by methods known in the art. The detectable change can also include expression of reporter genes/molecules such as Green Florescent Protein
15 (GFP) or various tags that are used to identify an expressed protein or any other cellular component that can be assayed.

In one embodiment, the invention features a kit containing a siNA molecule of the invention, which can be chemically-modified, that can be used to modulate the expression of a PARK1, PARK2, PARK5, and/or PARK7 target gene in a biological
20 system, including, for example, in a cell, tissue, or organism. In another embodiment, the invention features a kit containing more than one siNA molecule of the invention, which can be chemically-modified, that can be used to modulate the expression of more than one PARK1, PARK2, PARK5, and/or PARK7 target gene in a biological system, including, for example, in a cell, tissue, or organism.

25 In one embodiment, the invention features a cell containing one or more siNA molecules of the invention, which can be chemically-modified. In another embodiment, the cell containing a siNA molecule of the invention is a mammalian cell. In yet another embodiment, the cell containing a siNA molecule of the invention is a human cell.

In one embodiment, the synthesis of a siNA molecule of the invention, which can be chemically-modified, comprises: (a) synthesis of two complementary strands of the siNA molecule; (b) annealing the two complementary strands together under conditions suitable to obtain a double-stranded siNA molecule. In another embodiment, synthesis
5 of the two complementary strands of the siNA molecule is by solid phase oligonucleotide synthesis. In yet another embodiment, synthesis of the two complementary strands of the siNA molecule is by solid phase tandem oligonucleotide synthesis.

In one embodiment, the invention features a method for synthesizing a siNA duplex molecule comprising: (a) synthesizing a first oligonucleotide sequence strand of
10 the siNA molecule, wherein the first oligonucleotide sequence strand comprises a cleavable linker molecule that can be used as a scaffold for the synthesis of the second oligonucleotide sequence strand of the siNA; (b) synthesizing the second oligonucleotide sequence strand of siNA on the scaffold of the first oligonucleotide sequence strand, wherein the second oligonucleotide sequence strand further comprises a chemical moiety
15 than can be used to purify the siNA duplex; (c) cleaving the linker molecule of (a) under conditions suitable for the two siNA oligonucleotide strands to hybridize and form a stable duplex; and (d) purifying the siNA duplex utilizing the chemical moiety of the second oligonucleotide sequence strand. In one embodiment, cleavage of the linker molecule in (c) above takes place during deprotection of the oligonucleotide, for example
20 under hydrolysis conditions using an alkylamine base such as methylamine. In one embodiment, the method of synthesis comprises solid phase synthesis on a solid support such as controlled pore glass (CPG) or polystyrene, wherein the first sequence of (a) is synthesized on a cleavable linker, such as a succinyl linker, using the solid support as a scaffold. The cleavable linker in (a) used as a scaffold for synthesizing the second strand
25 can comprise similar reactivity as the solid support derivatized linker, such that cleavage of the solid support derivatized linker and the cleavable linker of (a) takes place concomitantly. In another embodiment, the chemical moiety of (b) that can be used to isolate the attached oligonucleotide sequence comprises a trityl group, for example a dimethoxytrityl group, which can be employed in a trityl-on synthesis strategy as
30 described herein. In yet another embodiment, the chemical moiety, such as a dimethoxytrityl group, is removed during purification, for example, using acidic conditions.

In a further embodiment, the method for siNA synthesis is a solution phase synthesis or hybrid phase synthesis wherein both strands of the siNA duplex are synthesized in tandem using a cleavable linker attached to the first sequence which acts a scaffold for synthesis of the second sequence. Cleavage of the linker under conditions
5 suitable for hybridization of the separate siNA sequence strands results in formation of the double-stranded siNA molecule.

In another embodiment, the invention features a method for synthesizing a siNA duplex molecule comprising: (a) synthesizing one oligonucleotide sequence strand of the siNA molecule, wherein the sequence comprises a cleavable linker molecule that can
10 be used as a scaffold for the synthesis of another oligonucleotide sequence; (b) synthesizing a second oligonucleotide sequence having complementarity to the first sequence strand on the scaffold of (a), wherein the second sequence comprises the other strand of the double-stranded siNA molecule and wherein the second sequence further comprises a chemical moiety than can be used to isolate the attached oligonucleotide
15 sequence; (c) purifying the product of (b) utilizing the chemical moiety of the second oligonucleotide sequence strand under conditions suitable for isolating the full-length sequence comprising both siNA oligonucleotide strands connected by the cleavable linker and under conditions suitable for the two siNA oligonucleotide strands to hybridize and form a stable duplex. In one embodiment, cleavage of the linker molecule
20 in (c) above takes place during deprotection of the oligonucleotide, for example under hydrolysis conditions. In another embodiment, cleavage of the linker molecule in (c) above takes place after deprotection of the oligonucleotide. In another embodiment, the method of synthesis comprises solid phase synthesis on a solid support such as controlled pore glass (CPG) or polystyrene, wherein the first sequence of (a) is synthesized on a
25 cleavable linker, such as a succinyl linker, using the solid support as a scaffold. The cleavable linker in (a) used as a scaffold for synthesizing the second strand can comprise similar reactivity or differing reactivity as the solid support derivatized linker, such that cleavage of the solid support derivatized linker and the cleavable linker of (a) takes place either concomitantly or sequentially. In one embodiment, the chemical moiety of (b) that
30 can be used to isolate the attached oligonucleotide sequence comprises a trityl group, for example a dimethoxytrityl group.

In another embodiment, the invention features a method for making a double-stranded siNA molecule in a single synthetic process comprising: (a) synthesizing an oligonucleotide having a first and a second sequence, wherein the first sequence is complementary to the second sequence, and the first oligonucleotide sequence is linked
5 to the second sequence via a cleavable linker, and wherein a terminal 5'-protecting group, for example, a 5'-O-dimethoxytrityl group (5'-O-DMT) remains on the oligonucleotide having the second sequence; (b) deprotecting the oligonucleotide whereby the deprotection results in the cleavage of the linker joining the two oligonucleotide sequences; and (c) purifying the product of (b) under conditions suitable for isolating the
10 double-stranded siNA molecule, for example using a trityl-on synthesis strategy as described herein.

In another embodiment, the method of synthesis of siNA molecules of the invention comprises the teachings of Scaringe *et al.*, US Patent Nos. 5,889,136; 6,008,400; and 6,111,086, incorporated by reference herein in their entirety.

15 In one embodiment, the invention features siNA constructs that mediate RNAi against PARK1, PARK2, PARK5, and/or PARK7, wherein the siNA construct comprises one or more chemical modifications, for example, one or more chemical modifications having any of Formulae I-VII or any combination thereof that increases the nuclease resistance of the siNA construct.

20 In another embodiment, the invention features a method for generating siNA molecules with increased nuclease resistance comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased nuclease resistance.

25 In one embodiment, the invention features siNA constructs that mediate RNAi against PARK1, PARK2, PARK5, and/or PARK7, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the sense and antisense strands of the siNA construct.

In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the sense and antisense strands of the siNA molecule comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased binding affinity between the sense and antisense strands of the siNA molecule.

In one embodiment, the invention features siNA constructs that mediate RNAi against PARK1, PARK2, PARK5, and/or PARK7, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the antisense strand of the siNA construct and a complementary target RNA sequence within a cell.

In one embodiment, the invention features siNA constructs that mediate RNAi against PARK1, PARK2, PARK5, and/or PARK7, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the antisense strand of the siNA construct and a complementary target DNA sequence within a cell.

In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the antisense strand of the siNA molecule and a complementary target RNA sequence comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased binding affinity between the antisense strand of the siNA molecule and a complementary target RNA sequence.

In another embodiment, the invention features a method for generating siNA molecules with increased binding affinity between the antisense strand of the siNA molecule and a complementary target DNA sequence comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having increased binding affinity between the antisense strand of the siNA molecule and a complementary target DNA sequence.

In one embodiment, the invention features siNA constructs that mediate RNAi against PARK1, PARK2, PARK5, and/or PARK7, wherein the siNA construct comprises one or more chemical modifications described herein that modulate the polymerase activity of a cellular polymerase capable of generating additional
5 endogenous siNA molecules having sequence homology to the chemically-modified siNA construct.

In another embodiment, the invention features a method for generating siNA molecules capable of mediating increased polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology
10 to a chemically-modified siNA molecule comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules capable of mediating increased polymerase activity of a cellular polymerase capable of generating additional endogenous siNA molecules having sequence homology to the
15 chemically-modified siNA molecule.

In one embodiment, the invention features chemically-modified siNA constructs that mediate RNAi against PARK1, PARK2, PARK5, and/or PARK7 in a cell, wherein the chemical modifications do not significantly effect the interaction of siNA with a target RNA molecule, DNA molecule and/or proteins or other factors that are essential
20 for RNAi in a manner that would decrease the efficacy of RNAi mediated by such siNA constructs.

In another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against PARK1, PARK2, PARK5, and/or PARK7 comprising (a) introducing nucleotides having any of Formula I-VII or any
25 combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity.

In yet another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against PARK1, PARK2, PARK5, and/or
30 PARK7 target RNA comprising (a) introducing nucleotides having any of Formula I-VII

or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity against the target RNA.

5 In yet another embodiment, the invention features a method for generating siNA molecules with improved RNAi activity against PARK1, PARK2, PARK5, and/or PARK7 target DNA comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved RNAi activity against the target DNA.

10 In one embodiment, the invention features siNA constructs that mediate RNAi against PARK1, PARK2, PARK5, and/or PARK7, wherein the siNA construct comprises one or more chemical modifications described herein that modulates the cellular uptake of the siNA construct.

15 In another embodiment, the invention features a method for generating siNA molecules against PARK1, PARK2, PARK5, and/or PARK7 with improved cellular uptake comprising (a) introducing nucleotides having any of Formula I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved cellular uptake.

20 In one embodiment, the invention features siNA constructs that mediate RNAi against PARK1, PARK2, PARK5, and/or PARK7, wherein the siNA construct comprises one or more chemical modifications described herein that increases the bioavailability of the siNA construct, for example, by attaching polymeric conjugates such as polyethyleneglycol or equivalent conjugates that improve the pharmacokinetics
25 of the siNA construct, or by attaching conjugates that target specific tissue types or cell types *in vivo*. Non-limiting examples of such conjugates are described in Vargeese *et al.*, U.S. Serial No. 10/201,394 incorporated by reference herein.

In one embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability, comprising (a) introducing a

conjugate into the structure of a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability. Such conjugates can include ligands for cellular receptors, such as peptides derived from naturally occurring protein ligands; protein localization sequences, including cellular ZIP code sequences; antibodies; nucleic acid aptamers; vitamins and other co-factors, such as folate and N-acetylgalactosamine; polymers, such as polyethyleneglycol (PEG); phospholipids; cholesterol; polyamines, such as spermine or spermidine; and others.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence is chemically modified in a manner that it can no longer act as a guide sequence for efficiently mediating RNA interference and/or be recognized by cellular proteins that facilitate RNAi.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein the second sequence is designed or modified in a manner that prevents its entry into the RNAi pathway as a guide sequence or as a sequence that is complementary to a target nucleic acid (e.g., RNA) sequence. Such design or modifications are expected to enhance the activity of siNA and/or improve the specificity of siNA molecules of the invention. These modifications are also expected to minimize any off-target effects and/or associated toxicity.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence is incapable of acting as a guide sequence for mediating RNA interference.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence does not have a
5 terminal 5'-hydroxyl (5'-OH) or 5'-phosphate group.

In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence comprises a
10 terminal cap moiety at the 5'-end of said second sequence. In one embodiment, the terminal cap moiety comprises an inverted abasic, inverted deoxy abasic, inverted nucleotide moiety, a group shown in Figure 10, an alkyl or cycloalkyl group, a heterocycle, or any other group that prevents RNAi activity in which the second sequence serves as a guide sequence or template for RNAi.

15 In one embodiment, the invention features a double stranded short interfering nucleic acid (siNA) molecule that comprises a first nucleotide sequence complementary to a target RNA sequence or a portion thereof, and a second sequence having complementarity to said first sequence, wherein said second sequence comprises a terminal cap moiety at the 5'-end and 3'-end of said second sequence. In one
20 embodiment, each terminal cap moiety individually comprises an inverted abasic, inverted deoxy abasic, inverted nucleotide moiety, a group shown in Figure 10, an alkyl or cycloalkyl group, a heterocycle, or any other group that prevents RNAi activity in which the second sequence serves as a guide sequence or template for RNAi.

In one embodiment, the invention features a method for generating siNA
25 molecules of the invention with improved specificity for down regulating or inhibiting the expression of a target nucleic acid (e.g., a DNA or RNA such as a gene or its corresponding RNA), comprising (a) introducing one or more chemical modifications into the structure of a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved specificity. In
30 another embodiment, the chemical modification used to improve specificity comprises terminal cap modifications at the 5'-end, 3'-end, or both 5' and 3'-ends of the siNA

molecule. The terminal cap modifications can comprise, for example, structures shown in Figure 10 (e.g. inverted deoxyabasic moieties) or any other chemical modification that renders a portion of the siNA molecule (e.g. the sense strand) incapable of mediating RNA interference against an off target nucleic acid sequence. In a non-limiting example, a siNA molecule is designed such that only the antisense sequence of the siNA molecule can serve as a guide sequence for RISC mediated degradation of a corresponding target RNA sequence. This can be accomplished by rendering the sense sequence of the siNA inactive by introducing chemical modifications to the sense strand that preclude recognition of the sense strand as a guide sequence by RNAi machinery. In one embodiment, such chemical modifications comprise any chemical group at the 5'-end of the sense strand of the siNA, or any other group that serves to render the sense strand inactive as a guide sequence for mediating RNA interference. These modifications, for example, can result in a molecule where the 5'-end of the sense strand no longer has a free 5'-hydroxyl (5'-OH) or a free 5'-phosphate group (e.g., phosphate, diphosphate, triphosphate, cyclic phosphate etc.). Non-limiting examples of such siNA constructs are described herein, such as "Stab 9/10", "Stab 7/8", "Stab 7/19", "Stab 17/22", "Stab 23/24", and "Stab 24/25" chemistries and variants thereof (see Table IV) wherein the 5'-end and 3'-end of the sense strand of the siNA do not comprise a hydroxyl group or phosphate group.

In one embodiment, the invention features a method for generating siNA molecules of the invention with improved specificity for down regulating or inhibiting the expression of a target nucleic acid (e.g., a DNA or RNA such as a gene or its corresponding RNA), comprising introducing one or more chemical modifications into the structure of a siNA molecule that prevent a strand or portion of the siNA molecule from acting as a template or guide sequence for RNAi activity. In one embodiment, the inactive strand or sense region of the siNA molecule is the sense strand or sense region of the siNA molecule, i.e. the strand or region of the siNA that does not have complementarity to the target nucleic acid sequence. In one embodiment, such chemical modifications comprise any chemical group at the 5'-end of the sense strand or region of the siNA that does not comprise a 5'-hydroxyl (5'-OH) or 5'-phosphate group, or any other group that serves to render the sense strand or sense region inactive as a guide sequence for mediating RNA interference. Non-limiting examples of such siNA

constructs are described herein, such as “Stab 9/10”, “Stab 7/8”, “Stab 7/19”, “Stab 17/22”, “Stab 23/24”, and “Stab 24/25” chemistries and variants thereof (see Table IV) wherein the 5'-end and 3'-end of the sense strand of the siNA do not comprise a hydroxyl group or phosphate group.

5 In one embodiment, the invention features a method for screening siNA molecules that are active in mediating RNA interference against a target nucleic acid sequence comprising (a) generating a plurality of unmodified siNA molecules, (b) screening the siNA molecules of step (a) under conditions suitable for isolating siNA molecules that are active in mediating RNA interference against the target nucleic acid sequence, and
10 (c) introducing chemical modifications (e.g. chemical modifications as described herein or as otherwise known in the art) into the active siNA molecules of (b). In one embodiment, the method further comprises re-screening the chemically modified siNA molecules of step (c) under conditions suitable for isolating chemically modified siNA molecules that are active in mediating RNA interference against the target nucleic acid
15 sequence.

In one embodiment, the invention features a method for screening chemically modified siNA molecules that are active in mediating RNA interference against a target nucleic acid sequence comprising (a) generating a plurality of chemically modified siNA molecules (e.g. siNA molecules as described herein or as otherwise known in the art),
20 and (b) screening the siNA molecules of step (a) under conditions suitable for isolating chemically modified siNA molecules that are active in mediating RNA interference against the target nucleic acid sequence.

The term “ligand” refers to any compound or molecule, such as a drug, peptide, hormone, or neurotransmitter, that is capable of interacting with another compound, such
25 as a receptor, either directly or indirectly. The receptor that interacts with a ligand can be present on the surface of a cell or can alternately be an intercellular receptor. Interaction of the ligand with the receptor can result in a biochemical reaction, or can simply be a physical interaction or association.

In another embodiment, the invention features a method for generating siNA
30 molecules of the invention with improved bioavailability comprising (a) introducing an

excipient formulation to a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability. Such excipients include polymers such as cyclodextrins, lipids, cationic lipids, polyamines, phospholipids, nanoparticles, receptors, ligands, and others.

5 In another embodiment, the invention features a method for generating siNA molecules of the invention with improved bioavailability comprising (a) introducing nucleotides having any of Formulae I-VII or any combination thereof into a siNA molecule, and (b) assaying the siNA molecule of step (a) under conditions suitable for isolating siNA molecules having improved bioavailability.

10 In another embodiment, polyethylene glycol (PEG) can be covalently attached to siNA compounds of the present invention. The attached PEG can be any molecular weight, preferably from about 2,000 to about 50,000 daltons (Da).

 The present invention can be used alone or as a component of a kit having at least one of the reagents necessary to carry out the *in vitro* or *in vivo* introduction of RNA to
15 test samples and/or subjects. For example, preferred components of the kit include a siNA molecule of the invention and a vehicle that promotes introduction of the siNA into cells of interest as described herein (e.g., using lipids and other methods of transfection known in the art, see for example Beigelman *et al.*, US 6,395,713). The kit can be used for target validation, such as in determining gene function and/or activity, or in drug
20 optimization, and in drug discovery (see for example Usman *et al.*, USSN 60/402,996). Such a kit can also include instructions to allow a user of the kit to practice the invention.

 The term "short interfering nucleic acid", "siNA", "short interfering RNA", "siRNA", "short interfering nucleic acid molecule", "short interfering oligonucleotide molecule", or "chemically-modified short interfering nucleic acid molecule" as used
25 herein refers to any nucleic acid molecule capable of inhibiting or down regulating gene expression or viral replication, for example by mediating RNA interference "RNAi" or gene silencing in a sequence-specific manner; see for example Zamore *et al.*, 2000, *Cell*, 101, 25-33; Bass, 2001, *Nature*, 411, 428-429; Elbashir *et al.*, 2001, *Nature*, 411, 494-498; and Kreutzer *et al.*, International PCT Publication No. WO 00/44895; Zernicka-
30 Goetz *et al.*, International PCT Publication No. WO 01/36646; Fire, International PCT

Publication No. WO 99/32619; Plaetinck *et al.*, International PCT Publication No. WO 00/01846; Mello and Fire, International PCT Publication No. WO 01/29058; Deschamps-Depaillette, International PCT Publication No. WO 99/07409; and Li *et al.*, International PCT Publication No. WO 00/44914; Allshire, 2002, *Science*, 297, 1818-1819; Volpe *et al.*, 2002, *Science*, 297, 1833-1837; Jenuwein, 2002, *Science*, 297, 2215-2218; and Hall *et al.*, 2002, *Science*, 297, 2232-2237; Hutvagner and Zamore, 2002, *Science*, 297, 2056-60; McManus *et al.*, 2002, *RNA*, 8, 842-850; Reinhart *et al.*, 2002, *Gene & Dev.*, 16, 1616-1626; and Reinhart & Bartel, 2002, *Science*, 297, 1831). Non limiting examples of siNA molecules of the invention are shown in **Figures 4-6**, and **Tables II and III** herein. For example the siNA can be a double-stranded polynucleotide molecule comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The siNA can be assembled from two separate oligonucleotides, where one strand is the sense strand and the other is the antisense strand, wherein the antisense and sense strands are self-complementary (i.e. each strand comprises nucleotide sequence that is complementary to nucleotide sequence in the other strand; such as where the antisense strand and sense strand form a duplex or double stranded structure, for example wherein the double stranded region is about 19 base pairs); the antisense strand comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense strand comprises nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. Alternatively, the siNA is assembled from a single oligonucleotide, where the self-complementary sense and antisense regions of the siNA are linked by means of a nucleic acid based or non-nucleic acid-based linker(s). The siNA can be a polynucleotide with a duplex, asymmetric duplex, hairpin or asymmetric hairpin secondary structure, having self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a separate target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The siNA can be a circular single-stranded polynucleotide having two or more loop structures

and a stem comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof, and wherein the circular polynucleotide can be processed either *in vivo* or *in vitro* to generate an active siNA molecule capable of mediating RNAi. The siNA can also comprise a single stranded polynucleotide having nucleotide sequence complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof (for example, where such siNA molecule does not require the presence within the siNA molecule of nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof), wherein the single stranded polynucleotide can further comprise a terminal phosphate group, such as a 5'-phosphate (see for example Martinez *et al.*, 2002, *Cell.*, 110, 563-574 and Schwarz *et al.*, 2002, *Molecular Cell*, 10, 537-568), or 5',3'-diphosphate. In certain embodiments, the siNA molecule of the invention comprises separate sense and antisense sequences or regions, wherein the sense and antisense regions are covalently linked by nucleotide or non-nucleotide linker molecules as is known in the art, or are alternately non-covalently linked by ionic interactions, hydrogen bonding, van der Waals interactions, hydrophobic interactions, and/or stacking interactions. In certain embodiments, the siNA molecules of the invention comprise nucleotide sequence that is complementary to nucleotide sequence of a target gene. In another embodiment, the siNA molecule of the invention interacts with nucleotide sequence of a target gene in a manner that causes inhibition of expression of the target gene. As used herein, siNA molecules need not be limited to those molecules containing only RNA, but further encompasses chemically-modified nucleotides and non-nucleotides. In certain embodiments, the short interfering nucleic acid molecules of the invention lack 2'-hydroxy (2'-OH) containing nucleotides. Applicant describes in certain embodiments short interfering nucleic acids that do not require the presence of nucleotides having a 2'-hydroxy group for mediating RNAi and as such, short interfering nucleic acid molecules of the invention optionally do not include any ribonucleotides (e.g., nucleotides having a 2'-OH group). Such siNA molecules that do not require the presence of ribonucleotides within the siNA molecule to support RNAi can however have an attached linker or linkers or other attached or associated groups, moieties, or

chains containing one or more nucleotides with 2'-OH groups. Optionally, siNA molecules can comprise ribonucleotides at about 5, 10, 20, 30, 40, or 50% of the nucleotide positions. The modified short interfering nucleic acid molecules of the invention can also be referred to as short interfering modified oligonucleotides "siMON."

5 As used herein, the term siNA is meant to be equivalent to other terms used to describe nucleic acid molecules that are capable of mediating sequence specific RNAi, for example short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), short hairpin RNA (shRNA), short interfering oligonucleotide, short interfering nucleic acid, short interfering modified oligonucleotide, chemically-modified
10 siRNA, post-transcriptional gene silencing RNA (ptgsRNA), and others. In addition, as used herein, the term RNAi is meant to be equivalent to other terms used to describe sequence specific RNA interference, such as post transcriptional gene silencing, translational inhibition, or epigenetics. For example, siNA molecules of the invention can be used to epigenetically silence genes at both the post-transcriptional level or the
15 pre-transcriptional level. In a non-limiting example, epigenetic regulation of gene expression by siNA molecules of the invention can result from siNA mediated modification of chromatin structure or methylation pattern to alter gene expression (see, for example, Verdel *et al.*, 2004, *Science*, 303, 672-676; Pal-Bhadra *et al.*, 2004, *Science*, 303, 669-672; Allshire, 2002, *Science*, 297, 1818-1819; Volpe *et al.*, 2002, *Science*, 297, 1833-1837; Jenuwein, 2002, *Science*, 297, 2215-2218; and Hall *et al.*, 2002, *Science*,
20 297, 2232-2237).

In one embodiment, a siNA molecule of the invention is a duplex forming oligonucleotide "DFO", (see for example Figures 14-15 and Vaish *et al.*, USSN 10/727,780 filed December 3, 2003 and International PCT Application No. filed May 24,
25 2004).

In one embodiment, a siNA molecule of the invention is a multifunctional siNA, (see for example Figures 16-22 and Jadhav *et al.*, USSN 60/543,480 filed February 10, 2004 and International PCT Application No. filed May 24, 2004). The multifunctional siNA of the invention can comprise sequence targeting, for example, two regions of
30 PARK1, PARK2, PARK5, and/or PARK7 RNA (see for example target sequences in Tables II and III).

By "asymmetric hairpin" as used herein is meant a linear siNA molecule comprising an antisense region, a loop portion that can comprise nucleotides or non-nucleotides, and a sense region that comprises fewer nucleotides than the antisense region to the extent that the sense region has enough complementary nucleotides to base pair with the antisense region and form a duplex with loop. For example, an asymmetric hairpin siNA molecule of the invention can comprise an antisense region having length sufficient to mediate RNAi in a cell or in vitro system (e.g. about 19 to about 22, or about 19, 20, 21, or 22 nucleotides) and a loop region comprising about 4 to about 8 (e.g., about 4, 5, 6, 7, or 8) nucleotides, and a sense region having about 3 to about 18 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18) nucleotides that are complementary to the antisense region. The asymmetric hairpin siNA molecule can also comprise a 5'-terminal phosphate group that can be chemically modified. The loop portion of the asymmetric hairpin siNA molecule can comprise nucleotides, non-nucleotides, linker molecules, or conjugate molecules as described herein.

By "asymmetric duplex" as used herein is meant a siNA molecule having two separate strands comprising a sense region and an antisense region, wherein the sense region comprises fewer nucleotides than the antisense region to the extent that the sense region has enough complementary nucleotides to base pair with the antisense region and form a duplex. For example, an asymmetric duplex siNA molecule of the invention can comprise an antisense region having length sufficient to mediate RNAi in a cell or in vitro system e.g. about 19 to about 22 (e.g. about 19, 20, 21, or 22) nucleotides and a sense region having about 3 to about 18 (e.g., about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18) nucleotides that are complementary to the antisense region.

By "modulate" is meant that the expression of the gene, or level of RNA molecule or equivalent RNA molecules encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits is up regulated or down regulated, such that expression, level, or activity is greater than or less than that observed in the absence of the modulator. For example, the term "modulate" can mean "inhibit," but the use of the word "modulate" is not limited to this definition.

By "inhibit", "down-regulate", or "reduce", it is meant that the expression of the gene, or level of RNA molecules or equivalent RNA molecules encoding one or more

proteins or protein subunits, or activity of one or more proteins or protein subunits, is reduced below that observed in the absence of the nucleic acid molecules (e.g., siNA) of the invention. In one embodiment, inhibition, down-regulation or reduction with an siNA molecule is below that level observed in the presence of an inactive or attenuated molecule. In another embodiment, inhibition, down-regulation, or reduction with siNA molecules is below that level observed in the presence of, for example, an siNA molecule with scrambled sequence or with mismatches. In another embodiment, inhibition, down-regulation, or reduction of gene expression with a nucleic acid molecule of the instant invention is greater in the presence of the nucleic acid molecule than in its absence.

By "gene", or "target gene", is meant, a nucleic acid that encodes an RNA, for example, nucleic acid sequences including, but not limited to, structural genes encoding a polypeptide. A gene or target gene can also encode a functional RNA (fRNA) or non-coding RNA (ncRNA), such as small temporal RNA (stRNA), micro RNA (miRNA), small nuclear RNA (snRNA), short interfering RNA (siRNA), small nucleolar RNA (snoRNA), ribosomal RNA (rRNA), transfer RNA (tRNA) and precursor RNAs thereof. Such non-coding RNAs can serve as target nucleic acid molecules for siNA mediated RNA interference in modulating the activity of fRNA or ncRNA involved in functional or regulatory cellular processes. Abberant fRNA or ncRNA activity leading to disease can therefore be modulated by siNA molecules of the invention. siNA molecules targeting fRNA and ncRNA can also be used to manipulate or alter the genotype or phenotype of an organism or cell, by intervening in cellular processes such as genetic imprinting, transcription, translation, or nucleic acid processing (e.g., transamination, methylation etc.). The target gene can be a gene derived from a cell, an endogenous gene, a transgene, or exogenous genes such as genes of a pathogen, for example a virus, which is present in the cell after infection thereof. The cell containing the target gene can be derived from or contained in any organism, for example a plant, animal, protozoan, virus, bacterium, or fungus. Non-limiting examples of plants include monocots, dicots, or gymnosperms. Non-limiting examples of animals include vertebrates or invertebrates. Non-limiting examples of fungi include molds or yeasts. For a review, see for example Snyder and Gerstein, 2003, *Science*, 300, 258-260.

By “non-canonical base pair” is meant any non-Watson Crick base pair, such as mismatches and/or wobble base pairs, including flipped mismatches, single hydrogen bond mismatches, trans-type mismatches, triple base interactions, and quadruple base interactions. Non-limiting examples of such non-canonical base pairs include, but are not limited to, AC reverse Hoogsteen, AC wobble, AU reverse Hoogsteen, GU wobble, AA N7 amino, CC 2-carbonyl-amino(H1)-N3-amino(H2), GA sheared, UC 4-carbonyl-amino, UU imino-carbonyl, AC reverse wobble, AU Hoogsteen, AU reverse Watson Crick, CG reverse Watson Crick, GC N3-amino-amino N3, AA N1-amino symmetric, AA N7-amino symmetric, GA N7-N1 amino-carbonyl, GA+ carbonyl-amino N7-N1, GG N1-carbonyl symmetric, GG N3-amino symmetric, CC carbonyl-amino symmetric, CC N3-amino symmetric, UU 2-carbonyl-imino symmetric, UU 4-carbonyl-imino symmetric, AA amino-N3, AA N1-amino, AC amino 2-carbonyl, AC N3-amino, AC N7-amino, AU amino-4-carbonyl, AU N1-imino, AU N3-imino, AU N7-imino, CC carbonyl-amino, GA amino-N1, GA amino-N7, GA carbonyl-amino, GA N3-amino, GC amino-N3, GC carbonyl-amino, GC N3-amino, GC N7-amino, GG amino-N7, GG carbonyl-imino, GG N7-amino, GU amino-2-carbonyl, GU carbonyl-imino, GU imino-2-carbonyl, GU N7-imino, psiU imino-2-carbonyl, UC 4-carbonyl-amino, UC imino-carbonyl, UU imino-4-carbonyl, AC C2-H-N3, GA carbonyl-C2-H, UU imino-4-carbonyl 2 carbonyl-C5-H, AC amino(A) N3(C)-carbonyl, GC imino amino-carbonyl, Gpsi imino-2-carbonyl amino-2-carbonyl, and GU imino amino-2-carbonyl base pairs.

By “PARK” as used herein is meant, any gene, RNA transcript, or protein (e.g., PARK1, PARK2, PARK5, and/or PARK7) associated with the development, maintenance, or progression of Parkinson disease.

By “PARK1” or “SNCA” as used herein is meant, any synuclein (e.g., alpha-synuclein, SNCA) or mutant synuclein protein, peptide, or polypeptide having synuclein activity, such as encoded by SNCA or SCNB Genbank Accession Nos. shown in Table I. The term PARK1 also refers to nucleic acid sequences encoding any synuclein protein, peptide, or polypeptide having synuclein activity or mutant synuclein nucleic acid sequences encoding any mutant synuclein protein, peptide, or polypeptide having mutant synuclein activity.

By "PARK2" as used herein is meant, any PARK2 protein, peptide, or polypeptide having PARK2 activity (characterized by mutations in the parkin gene which are associated with autosomal recessive juvenile parkinsonism), such as encoded by PARK2 Genbank Accession Nos. shown in Table I.

5 By "PARK5" as used herein is meant, any PARK5 protein, peptide, or polypeptide having PARK5 activity (characterized by mutations in the UCH-L1 gene encoding ubiquitin carboxy-terminal hydrolase L1), such as encoded by PARK5 Genbank Accession Nos. shown in Table I.

10 By "PARK7" as used herein is meant, any PARK7 protein, peptide, or polypeptide having PARK7 activity (characterized by mutations in the DJ-1 gene), such as encoded by PARK7 Genbank Accession Nos. shown in Table I.

By "mutant" as used herein is meant, any polynucleotide or polypeptide sequence that differs from a wild type polynucleotide or polypeptide sequence. The mutant polynucleotide or polypeptide sequence can be associated with a disease state, such as
15 Parkinson's disease.

By "homologous sequence" is meant, a nucleotide sequence that is shared by one or more polynucleotide sequences, such as genes, gene transcripts and/or non-coding polynucleotides. For example, a homologous sequence can be a nucleotide sequence that is shared by two or more genes encoding related but different proteins, such as different
20 members of a gene family, different protein epitopes, different protein isoforms or completely divergent genes, such as a cytokine and its corresponding receptors. A homologous sequence can be a nucleotide sequence that is shared by two or more non-coding polynucleotides, such as noncoding DNA or RNA, regulatory sequences, introns, and sites of transcriptional control or regulation. Homologous sequences can also
25 include conserved sequence regions shared by more than one polynucleotide sequence. Homology does not need to be perfect homology (e.g., 100%), as partially homologous sequences are also contemplated by the instant invention (e.g., 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80% etc.).

By "conserved sequence region" is meant, a nucleotide sequence of one or more regions in a polynucleotide does not vary significantly between generations or from one biological system or organism to another biological system or organism. The polynucleotide can include both coding and non-coding DNA and RNA.

- 5 By "sense region" is meant a nucleotide sequence of a siNA molecule having complementarity to an antisense region of the siNA molecule. In addition, the sense region of a siNA molecule can comprise a nucleic acid sequence having homology with a target nucleic acid sequence.

- 10 By "antisense region" is meant a nucleotide sequence of a siNA molecule having complementarity to a target nucleic acid sequence. In addition, the antisense region of a siNA molecule can optionally comprise a nucleic acid sequence having complementarity to a sense region of the siNA molecule.

By "target nucleic acid" is meant any nucleic acid sequence whose expression or activity is to be modulated. The target nucleic acid can be DNA or RNA.

- 15 By "complementarity" is meant that a nucleic acid can form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick or other non-traditional types. In reference to the nucleic molecules of the present invention, the binding free energy for a nucleic acid molecule with its complementary sequence is sufficient to allow the relevant function of the nucleic acid to proceed, e.g., RNAi activity.
- 20 Determination of binding free energies for nucleic acid molecules is well known in the art (see, e.g., Turner *et al.*, 1987, *CSH Symp. Quant. Biol.* LII pp.123-133; Frier *et al.*, 1986, *Proc. Nat. Acad. Sci. USA* 83:9373-9377; Turner *et al.*, 1987, *J. Am. Chem. Soc.* 109:3783-3785). A percent complementarity indicates the percentage of contiguous residues in a nucleic acid molecule that can form hydrogen bonds (e.g., Watson-Crick
- 25 base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9, or 10 nucleotides out of a total of 10 nucleotides in the first oligonucleotide being based paired to a second nucleic acid sequence having 10 nucleotides represents 50%, 60%, 70%, 80%, 90%, and 100% complementary respectively). "Perfectly complementary" means that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same
- 30 number of contiguous residues in a second nucleic acid sequence.

In one embodiment, siNA molecules of the invention that down regulate or reduce PARK1, PARK2, PARK5, and/or PARK7 gene expression are used for preventing or reducing Parkinson's disease, Alzheimer's disease, and/or dementia in a subject. In one embodiment, the siNA molecules of the invention that down regulate or reduce PARK1,
5 PARK2, PARK5, and/or PARK7 gene expression are used for treating or preventing Parkinson's disease, Alzheimer's disease, and/or dementia in a subject.

In one embodiment of the present invention, each sequence of a siNA molecule of the invention is independently about 18 to about 24 nucleotides in length, in specific embodiments about 18, 19, 20, 21, 22, 23, or 24 nucleotides in length. In another
10 embodiment, the siNA duplexes of the invention independently comprise about 17 to about 23 base pairs (e.g., about 17, 18, 19, 20, 21, 22, or 23). In yet another embodiment, siNA molecules of the invention comprising hairpin or circular structures are about 35 to about 55 (e.g., about 35, 40, 45, 50 or 55) nucleotides in length, or about 38 to about 44 (e.g., about 38, 39, 40, 41, 42, 43, or 44) nucleotides in length and comprising about 16
15 to about 22 (e.g., about 16, 17, 18, 19, 20, 21 or 22) base pairs. Exemplary siNA molecules of the invention are shown in Table II. Exemplary synthetic siNA molecules of the invention are shown in Table III and/or Figures 4-5.

As used herein "cell" is used in its usual biological sense, and does not refer to an entire multicellular organism, e.g., specifically does not refer to a human. The cell can
20 be present in an organism, e.g., birds, plants and mammals such as humans, cows, sheep, apes, monkeys, swine, dogs, and cats. The cell can be prokaryotic (e.g., bacterial cell) or eukaryotic (e.g., mammalian or plant cell). The cell can be of somatic or germ line origin, totipotent or pluripotent, dividing or non-dividing. The cell can also be derived from or can comprise a gamete or embryo, a stem cell, or a fully differentiated cell.

25 The siNA molecules of the invention are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells or tissues. The nucleic acid or nucleic acid complexes can be locally administered to relevant tissues *ex vivo*, or *in vivo* through direct dermal application, transdermal application, or injection, with or without their incorporation in biopolymers. In
30 particular embodiments, the nucleic acid molecules of the invention comprise sequences shown in Tables II-III and/or Figures 4-5. Examples of such nucleic acid molecules

consist essentially of sequences defined in these tables and figures. Furthermore, the chemically modified constructs described in Table IV can be applied to any siNA sequence of the invention.

5 In another aspect, the invention provides mammalian cells containing one or more siNA molecules of this invention. The one or more siNA molecules can independently be targeted to the same or different sites.

By "RNA" is meant a molecule comprising at least one ribonucleotide residue. By "ribonucleotide" is meant a nucleotide with a hydroxyl group at the 2' position of a β -D-ribofuranose moiety. The terms include double-stranded RNA, single-stranded RNA,
10 isolated RNA such as partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA, as well as altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of the siNA or internally, for example at one or more nucleotides of the RNA.
15 Nucleotides in the RNA molecules of the instant invention can also comprise non-standard nucleotides, such as non-naturally occurring nucleotides or chemically synthesized nucleotides or deoxynucleotides. These altered RNAs can be referred to as analogs or analogs of naturally-occurring RNA.

By "subject" is meant an organism, which is a donor or recipient of explanted cells
20 or the cells themselves. "Subject" also refers to an organism to which the nucleic acid molecules of the invention can be administered. A subject can be a mammal or mammalian cells, including a human or human cells.

The term "phosphorothioate" as used herein refers to an internucleotide linkage having Formula I, wherein Z and/or W comprise a sulfur atom. Hence, the term
25 phosphorothioate refers to both phosphorothioate and phosphorodithioate internucleotide linkages.

The term "phosphonoacetate" as used herein refers to an internucleotide linkage having Formula I, wherein Z and/or W comprise an acetyl or protected acetyl group.

The term "thiophosphonoacetate" as used herein refers to an internucleotide linkage having Formula I, wherein Z comprises an acetyl or protected acetyl group and W comprises a sulfur atom or alternately W comprises an acetyl or protected acetyl group and Z comprises a sulfur atom.

5 The term "universal base" as used herein refers to nucleotide base analogs that form base pairs with each of the natural DNA/RNA bases with little discrimination between them. Non-limiting examples of universal bases include C-phenyl, C-naphthyl and other aromatic derivatives, inosine, azole carboxamides, and nitroazole derivatives such as 3-nitropyrrole, 4-nitroindole, 5-nitroindole, and 6-nitroindole as known in the art
10 (see for example Loakes, 2001, *Nucleic Acids Research*, 29, 2437-2447).

The term "acyclic nucleotide" as used herein refers to any nucleotide having an acyclic ribose sugar, for example where any of the ribose carbons (C1, C2, C3, C4, or C5), are independently or in combination absent from the nucleotide.

15 The nucleic acid molecules of the instant invention, individually, or in combination or in conjunction with other drugs, can be used to for preventing or treating Parkinson's disease, Alzheimer's disease, and/or dementia in a subject or organism. In one embodiment, siNA molecules of the invention are used in combination with neuroprotective agents (e.g., pyridoxine) to treat or prevent Parkinson's disease, Alzheimer's disease, and/or dementia in a subject or organism.

20 In one embodiment, the invention features a method for treating or preventing a disease or condition in a subject, wherein the disease or condition is related to a neurodegenerative process or condition, comprising administering to the subject a siNA molecule of the invention under conditions suitable for the treatment or prevention of the disease or condition in the subject, alone or in conjunction with one or more other
25 therapeutic compounds. In another embodiment, the disease or condition comprises neurodegenerative diseases, disorders, or conditions including Parkinson's disease, Alzheimers disease, dementia, and any other diseases or conditions that are related to or will respond to the levels of PARK1, PARK2, PARK5, and/or PARK7 in a cell or tissue, alone or in combination with other therapies.

In one embodiment, the invention features a method for treating or preventing Parkinson's disease in a subject, comprising administering to the subject a siNA molecule of the invention under conditions suitable for the treatment or prevention of Parkinson's disease in the subject, alone or in conjunction with one or more other
5 therapeutic compounds. In another embodiment, the Parkinson's disease comprises familial Parkinson's disease and/or dementia associated therewith.

In one embodiment, the invention features a method for treating or preventing Alzheimer's disease in a subject, comprising administering to the subject a siNA molecule of the invention under conditions suitable for the treatment or prevention of
10 Alzheimer's disease in the subject, alone or in conjunction with one or more other therapeutic compounds. In another embodiment, the Alzheimer's disease comprises familial Alzheimer's disease and/or dementia associated therewith.

For example, the siNA molecules can be administered to a subject or can be administered to other appropriate cells evident to those skilled in the art, individually or
15 in combination with one or more drugs under conditions suitable for the treatment.

In one embodiment, the invention features an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the invention, in a manner which allows expression of the siNA molecule. For example, the vector can contain sequence(s) encoding both strands of a siNA molecule comprising a duplex. The vector
20 can also contain sequence(s) encoding a single nucleic acid molecule that is self-complementary and thus forms a siNA molecule. Non-limiting examples of such expression vectors are described in Paul *et al.*, 2002, *Nature Biotechnology*, 19, 505; Miyagishi and Taira, 2002, *Nature Biotechnology*, 19, 497; Lee *et al.*, 2002, *Nature Biotechnology*, 19, 500; and Novina *et al.*, 2002, *Nature Medicine*, advance online
25 publication doi:10.1038/nm725.

In another embodiment, the invention features a mammalian cell, for example, a human cell, including an expression vector of the invention.

In yet another embodiment, the expression vector of the invention comprises a sequence for a siNA molecule having complementarity to a RNA molecule referred to by a Genbank Accession numbers, for example Genbank Accession Nos. shown in Table I.

5 In one embodiment, an expression vector of the invention comprises a nucleic acid sequence encoding two or more siNA molecules, which can be the same or different.

In another aspect of the invention, siNA molecules that interact with target RNA molecules and down-regulate gene encoding target RNA molecules (for example target RNA molecules referred to by Genbank Accession numbers herein) are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors can be
10 DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. The recombinant vectors capable of expressing the siNA molecules can be delivered as described herein, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of siNA molecules. Such vectors can be repeatedly
15 administered as necessary. Once expressed, the siNA molecules bind and down-regulate gene function or expression via RNA interference (RNAi). Delivery of siNA expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from a subject followed by reintroduction into the subject, or by any other means that would allow for introduction into the desired
20 target cell.

By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

25

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a non-limiting example of a scheme for the synthesis of siNA molecules. The complementary siNA sequence strands, strand 1 and strand 2, are synthesized in tandem and are connected by a cleavable linkage, such as a nucleotide succinate or abasic succinate, which can be the same or different from the cleavable

linker used for solid phase synthesis on a solid support. The synthesis can be either solid phase or solution phase, in the example shown, the synthesis is a solid phase synthesis. The synthesis is performed such that a protecting group, such as a dimethoxytrityl group, remains intact on the terminal nucleotide of the tandem oligonucleotide. Upon cleavage
5 and deprotection of the oligonucleotide, the two siNA strands spontaneously hybridize to form a siNA duplex, which allows the purification of the duplex by utilizing the properties of the terminal protecting group, for example by applying a trityl on purification method wherein only duplexes/oligonucleotides with the terminal protecting group are isolated.

10 **Figure 2** shows a MALDI-TOF mass spectrum of a purified siNA duplex synthesized by a method of the invention. The two peaks shown correspond to the predicted mass of the separate siNA sequence strands. This result demonstrates that the siNA duplex generated from tandem synthesis can be purified as a single entity using a simple trityl-on purification methodology.

15 **Figure 3** shows a non-limiting proposed mechanistic representation of target RNA degradation involved in RNAi. Double-stranded RNA (dsRNA), which is generated by RNA-dependent RNA polymerase (RdRP) from foreign single-stranded RNA, for example viral, transposon, or other exogenous RNA, activates the DICER enzyme that in turn generates siNA duplexes. Alternately, synthetic or expressed siNA can be
20 introduced directly into a cell by appropriate means. An active siNA complex forms which recognizes a target RNA, resulting in degradation of the target RNA by the RISC endonuclease complex or in the synthesis of additional RNA by RNA-dependent RNA polymerase (RdRP), which can activate DICER and result in additional siNA molecules, thereby amplifying the RNAi response.

25 **Figure 4A-F** shows non-limiting examples of chemically-modified siNA constructs of the present invention. In the figure, N stands for any nucleotide (adenosine, guanosine, cytosine, uridine, or optionally thymidine, for example thymidine can be substituted in the overhanging regions designated by parenthesis (N N). Various modifications are shown for the sense and antisense strands of the siNA constructs.

Figure 4A: The sense strand comprises 21 nucleotides wherein the two terminal 3'-nucleotides are optionally base paired and wherein all nucleotides present are ribonucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein.

5 The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all nucleotides present are ribonucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide
10 linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the antisense strand.

Figure 4B: The sense strand comprises 21 nucleotides wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may
15 be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-
20 nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified
25 internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the sense and antisense strand.

Figure 4C: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and
30 wherein all pyrimidine nucleotides that may be present are 2'-O-methyl or 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise

ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that
5 may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the
10 antisense strand.

Figure 4D: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides,
15 deoxynucleotides, universal bases, or other chemical modifications described herein and wherein and all purine nucleotides that may be present are 2'-deoxy nucleotides. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, wherein all pyrimidine nucleotides that may be present are 2'-
20 deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown
25 as "s", optionally connects the (N N) nucleotides in the antisense strand.

Figure 4E: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides,
30 deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl

moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise
 5 ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the antisense strand.

Figure 4F: The sense strand comprises 21 nucleotides having 5'- and 3'- terminal
 10 cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein and wherein and all purine nucleotides that may be present are 2'-deoxy nucleotides. The
 15 antisense strand comprises 21 nucleotides, optionally having a 3'-terminal glyceryl moiety and wherein the two terminal 3'-nucleotides are optionally complementary to the target RNA sequence, and having one 3'-terminal phosphorothioate internucleotide linkage and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides and all purine nucleotides that may be present are 2'-deoxy
 20 nucleotides except for (N N) nucleotides, which can comprise ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. A modified internucleotide linkage, such as a phosphorothioate, phosphorodithioate or other modified internucleotide linkage as described herein, shown as "s", optionally connects the (N N) nucleotides in the antisense strand. The antisense strand of
 25 constructs A-F comprise sequence complementary to any target nucleic acid sequence of the invention. Furthermore, when a glyceryl moiety (L) is present at the 3'-end of the antisense strand for any construct shown in Figure 4 A-F, the modified internucleotide linkage is optional.

Figure 5A-F shows non-limiting examples of specific chemically-modified siNA
 30 sequences of the invention. A-F applies the chemical modifications described in Figure 4A-F to a PARK1 (SNCA) siNA sequence. Such chemical modifications can be applied

to any PARK1, PARK2, PARK5, and/or PARK7 sequence and/or PARK1, PARK2, PARK5, and/or PARK7 polymorphism sequence.

Figure 6 shows non-limiting examples of different siNA constructs of the invention. The examples shown (constructs 1, 2, and 3) have 19 representative base pairs; however, different embodiments of the invention include any number of base pairs described herein. Bracketed regions represent nucleotide overhangs, for example comprising about 1, 2, 3, or 4 nucleotides in length, preferably about 2 nucleotides. Constructs 1 and 2 can be used independently for RNAi activity. Construct 2 can comprise a polynucleotide or non-nucleotide linker, which can optionally be designed as a biodegradable linker. In one embodiment, the loop structure shown in construct 2 can comprise a biodegradable linker that results in the formation of construct 1 *in vivo* and/or *in vitro*. In another example, construct 3 can be used to generate construct 2 under the same principle wherein a linker is used to generate the active siNA construct 2 *in vivo* and/or *in vitro*, which can optionally utilize another biodegradable linker to generate the active siNA construct 1 *in vivo* and/or *in vitro*. As such, the stability and/or activity of the siNA constructs can be modulated based on the design of the siNA construct for use *in vivo* or *in vitro* and/or *in vitro*.

Figure 7A-C is a diagrammatic representation of a scheme utilized in generating an expression cassette to generate siNA hairpin constructs.

Figure 7A: A DNA oligomer is synthesized with a 5'-restriction site (R1) sequence followed by a region having sequence identical (sense region of siNA) to a predetermined PARK1, PARK2, PARK5, and/or PARK7 target sequence, wherein the sense region comprises, for example, about 19, 20, 21, or 22 nucleotides (N) in length, which is followed by a loop sequence of defined sequence (X), comprising, for example, about 3 to about 10 nucleotides.

Figure 7B: The synthetic construct is then extended by DNA polymerase to generate a hairpin structure having self-complementary sequence that will result in a siNA transcript having specificity for a PARK1, PARK2, PARK5, and/or PARK7 target sequence and having self-complementary sense and antisense regions.

Figure 7C: The construct is heated (for example to about 95°C) to linearize the sequence, thus allowing extension of a complementary second DNA strand using a primer to the 3'-restriction sequence of the first strand. The double-stranded DNA is then inserted into an appropriate vector for expression in cells. The construct can be designed
5 such that a 3'-terminal nucleotide overhang results from the transcription, for example by engineering restriction sites and/or utilizing a poly-U termination region as described in Paul *et al.*, 2002, *Nature Biotechnology*, 29, 505-508.

Figure 8A-C is a diagrammatic representation of a scheme utilized in generating an expression cassette to generate double-stranded siNA constructs.

10 **Figure 8A:** A DNA oligomer is synthesized with a 5'-restriction (R1) site sequence followed by a region having sequence identical (sense region of siNA) to a predetermined PARK1, PARK2, PARK5, and/or PARK7 target sequence, wherein the sense region comprises, for example, about 19, 20, 21, or 22 nucleotides (N) in length, and which is followed by a 3'-restriction site (R2) which is adjacent to a loop sequence of
15 defined sequence (X).

Figure 8B: The synthetic construct is then extended by DNA polymerase to generate a hairpin structure having self-complementary sequence.

Figure 8C: The construct is processed by restriction enzymes specific to R1 and R2 to generate a double-stranded DNA which is then inserted into an appropriate vector
20 for expression in cells. The transcription cassette is designed such that a U6 promoter region flanks each side of the dsDNA which generates the separate sense and antisense strands of the siNA. Poly T termination sequences can be added to the constructs to generate U overhangs in the resulting transcript.

Figure 9A-E is a diagrammatic representation of a method used to determine
25 target sites for siNA mediated RNAi within a particular target nucleic acid sequence, such as messenger RNA.

Figure 9A: A pool of siNA oligonucleotides are synthesized wherein the antisense region of the siNA constructs has complementarity to target sites across the target

nucleic acid sequence, and wherein the sense region comprises sequence complementary to the antisense region of the siNA.

Figure 9B&C: (Figure 9B) The sequences are pooled and are inserted into vectors such that (Figure 9C) transfection of a vector into cells results in the expression of the siNA.

Figure 9D: Cells are sorted based on phenotypic change that is associated with modulation of the target nucleic acid sequence.

Figure 9E: The siNA is isolated from the sorted cells and is sequenced to identify efficacious target sites within the target nucleic acid sequence.

Figure 10 shows non-limiting examples of different stabilization chemistries (1-10) that can be used, for example, to stabilize the 3'-end of siNA sequences of the invention, including (1) [3-3']-inverted deoxyribose; (2) deoxyribonucleotide; (3) [5'-3']-3'-deoxyribonucleotide; (4) [5'-3']-ribonucleotide; (5) [5'-3']-3'-O-methyl ribonucleotide; (6) 3'-glyceryl; (7) [3'-5']-3'-deoxyribonucleotide; (8) [3'-3']-deoxyribonucleotide; (9) [5'-2']-deoxyribonucleotide; and (10) [5-3']-dideoxyribonucleotide. In addition to modified and unmodified backbone chemistries indicated in the figure, these chemistries can be combined with different backbone modifications as described herein, for example, backbone modifications having Formula I. In addition, the 2'-deoxy nucleotide shown 5' to the terminal modifications shown can be another modified or unmodified nucleotide or non-nucleotide described herein, for example modifications having any of Formulae I-VII or any combination thereof.

Figure 11 shows a non-limiting example of a strategy used to identify chemically modified siNA constructs of the invention that are nuclease resistance while preserving the ability to mediate RNAi activity. Chemical modifications are introduced into the siNA construct based on educated design parameters (e.g. introducing 2'-modifications, base modifications, backbone modifications, terminal cap modifications etc). The modified construct is tested in an appropriate system (e.g. human serum for nuclease resistance, shown, or an animal model for PK/delivery parameters). In parallel, the siNA construct is tested for RNAi activity, for example in a cell culture system such as a

luciferase reporter assay). Lead siNA constructs are then identified which possess a particular characteristic while maintaining RNAi activity, and can be further modified and assayed once again. This same approach can be used to identify siNA-conjugate molecules with improved pharmacokinetic profiles, delivery, and RNAi activity.

5 **Figure 12** shows non-limiting examples of phosphorylated siNA molecules of the invention, including linear and duplex constructs and asymmetric derivatives thereof.

Figure 13 shows non-limiting examples of chemically modified terminal phosphate groups of the invention.

10 **Figure 14A** shows a non-limiting example of methodology used to design self complementary DFO constructs utilizing palidrome and/or repeat nucleic acid sequences that are identified in a target nucleic acid sequence. (i) A palindrome or repeat sequence is identified in a nucleic acid target sequence. (ii) A sequence is designed that is complementary to the target nucleic acid sequence and the palindrome sequence. (iii) An
15 inverse repeat sequence of the non-palindrome/repeat portion of the complementary sequence is appended to the 3'-end of the complementary sequence to generate a self complementary DFO molecule comprising sequence complementary to the nucleic acid target. (iv) The DFO molecule can self-assemble to form a double stranded oligonucleotide. **Figure 14B** shows a non-limiting representative example of a duplex forming oligonucleotide sequence. **Figure 14C** shows a non-limiting example of the self
20 assembly schematic of a representative duplex forming oligonucleotide sequence. **Figure 14D** shows a non-limiting example of the self assembly schematic of a representative duplex forming oligonucleotide sequence followed by interaction with a target nucleic acid sequence resulting in modulation of gene expression.

25 **Figure 15** shows a non-limiting example of the design of self complementary DFO constructs utilizing palidrome and/or repeat nucleic acid sequences that are incorporated into the DFO constructs that have sequence complementary to any target nucleic acid sequence of interest. Incorporation of these palindrome/repeat sequences allow the design of DFO constructs that form duplexes in which each strand is capable of mediating modulation of target gene expression, for example by RNAi. First, the target
30 sequence is identified. A complementary sequence is then generated in which nucleotide

or non-nucleotide modifications (shown as X or Y) are introduced into the complementary sequence that generate an artificial palindrome (shown as XYXYXY in the Figure). An inverse repeat of the non-palindrome/repeat complementary sequence is appended to the 3'-end of the complementary sequence to generate a self complementary

5 DFO comprising sequence complementary to the nucleic acid target. The DFO can self-assemble to form a double stranded oligonucleotide.

Figure 16 shows non-limiting examples of multifunctional siNA molecules of the invention comprising two separate polynucleotide sequences that are each capable of mediating RNAi directed cleavage of differing target nucleic acid sequences. Figure

10 16A shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first and second complementary regions are situated at the 3'-ends of each polynucleotide sequence in the multifunctional siNA.

15 The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. Figure 16B shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first and second complementary regions are situated at the 5'-ends of each polynucleotide sequence in the multifunctional siNA.

20 The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences.

25

Figure 17 shows non-limiting examples of multifunctional siNA molecules of the invention comprising a single polynucleotide sequence comprising distinct regions that are each capable of mediating RNAi directed cleavage of differing target nucleic acid sequences. Figure 17A shows a non-limiting example of a multifunctional siNA

30 molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a

second target nucleic acid sequence (complementary region 2), wherein the second complementary region is situated at the 3'-end of the polynucleotide sequence in the multifunctional siNA. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. **Figure 17B** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first complementary region is situated at the 5'-end of the polynucleotide sequence in the multifunctional siNA. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. In one embodiment, these multifunctional siNA constructs are processed in vivo or in vitro to generate multifunctional siNA constructs as shown in **Figure 16**.

Figure 18 shows non-limiting examples of multifunctional siNA molecules of the invention comprising two separate polynucleotide sequences that are each capable of mediating RNAi directed cleavage of differing target nucleic acid sequences and wherein the multifunctional siNA construct further comprises a self complementary, palindrome, or repeat region, thus enabling shorter bifunctional siNA constructs that can mediate RNA interference against differing target nucleic acid sequences. **Figure 18A** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first and second complementary regions are situated at the 3'-ends of each polynucleotide sequence in the multifunctional siNA, and wherein the first and second complementary regions further comprise a self complementary, palindrome, or repeat region. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. **Figure 18B** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary

to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first and second complementary regions are situated at the 5'-ends of each polynucleotide sequence in the multifunctional siNA, and wherein the first and second
5 complementary regions further comprise a self complementary, palindrome, or repeat region. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences.

Figure 19 shows non-limiting examples of multifunctional siNA molecules of the
10 invention comprising a single polynucleotide sequence comprising distinct regions that are each capable of mediating RNAi directed cleavage of differing target nucleic acid sequences and wherein the multifunctional siNA construct further comprises a self complementary, palindrome, or repeat region, thus enabling shorter bifunctional siNA constructs that can mediate RNA interference against differing target nucleic acid
15 sequences. **Figure 19A** shows a non-limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the second complementary region is situated at the 3'-end of the polynucleotide sequence in the
20 multifunctional siNA, and wherein the first and second complementary regions further comprise a self complementary, palindrome, or repeat region. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. **Figure 19B** shows a non-
25 limiting example of a multifunctional siNA molecule having a first region that is complementary to a first target nucleic acid sequence (complementary region 1) and a second region that is complementary to a second target nucleic acid sequence (complementary region 2), wherein the first complementary region is situated at the 5'-end of the polynucleotide sequence in the multifunctional siNA, and wherein the first
30 and second complementary regions further comprise a self complementary, palindrome, or repeat region. The dashed portions of each polynucleotide sequence of the multifunctional siNA construct have complementarity with regard to corresponding

portions of the siNA duplex, but do not have complementarity to the target nucleic acid sequences. In one embodiment, these multifunctional siNA constructs are processed in vivo or in vitro to generate multifunctional siNA constructs as shown in Figure 18.

Figure 20 shows a non-limiting example of how multifunctional siNA molecules of the invention can target two separate target nucleic acid molecules, such as separate RNA molecules encoding differing proteins, for example a cytokine and its corresponding receptor, differing viral strains, a virus and a cellular protein involved in viral infection or replication, or differing proteins involved in a common or divergent biologic pathway that is implicated in the maintenance of progression of disease. Each strand of the multifunctional siNA construct comprises a region having complementarity to separate target nucleic acid molecules. The multifunctional siNA molecule is designed such that each strand of the siNA can be utilized by the RISC complex to initiate RNA interference mediated cleavage of its corresponding target. These design parameters can include destabilization of each end of the siNA construct (see for example Schwarz *et al.*, 2003, *Cell*, 115, 199-208). Such destabilization can be accomplished for example by using guanosine-cytidine base pairs, alternate base pairs (e.g., wobbles), or destabilizing chemically modified nucleotides at terminal nucleotide positions as is known in the art.

Figure 21 shows a non-limiting example of how multifunctional siNA molecules of the invention can target two separate target nucleic acid sequences within the same target nucleic acid molecule, such as alternate coding regions of a RNA, coding and non-coding regions of a RNA, or alternate splice variant regions of a RNA. Each strand of the multifunctional siNA construct comprises a region having complementarity to the separate regions of the target nucleic acid molecule. The multifunctional siNA molecule is designed such that each strand of the siNA can be utilized by the RISC complex to initiate RNA interference mediated cleavage of its corresponding target region. These design parameters can include destabilization of each end of the siNA construct (see for example Schwarz *et al.*, 2003, *Cell*, 115, 199-208). Such destabilization can be accomplished for example by using guanosine-cytidine base pairs, alternate base pairs (e.g., wobbles), or destabilizing chemically modified nucleotides at terminal nucleotide positions as is known in the art.

DETAILED DESCRIPTION OF THE INVENTION

Mechanism of Action of Nucleic Acid Molecules of the Invention

The discussion that follows discusses the proposed mechanism of RNA interference mediated by short interfering RNA as is presently known, and is not meant to be limiting and is not an admission of prior art. Applicant demonstrates herein that chemically-modified short interfering nucleic acids possess similar or improved capacity to mediate RNAi as do siRNA molecules and are expected to possess improved stability and activity *in vivo*; therefore, this discussion is not meant to be limiting only to siRNA and can be applied to siNA as a whole. By "improved capacity to mediate RNAi" or "improved RNAi activity" is meant to include RNAi activity measured *in vitro* and/or *in vivo* where the RNAi activity is a reflection of both the ability of the siNA to mediate RNAi and the stability of the siNAs of the invention. In this invention, the product of these activities can be increased *in vitro* and/or *in vivo* compared to an all RNA siRNA or a siNA containing a plurality of ribonucleotides. In some cases, the activity or stability of the siNA molecule can be decreased (i.e., less than ten-fold), but the overall activity of the siNA molecule is enhanced *in vitro* and/or *in vivo*.

RNA interference refers to the process of sequence specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs) (Fire *et al.*, 1998, *Nature*, 391, 806). The corresponding process in plants is commonly referred to as post-transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of post-transcriptional gene silencing is thought to be an evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes which is commonly shared by diverse flora and phyla (Fire *et al.*, 1999, *Trends Genet.*, 15, 358). Such protection from foreign gene expression may have evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA. The presence of dsRNA in cells triggers the RNAi response through a mechanism that has yet to be fully characterized. This mechanism appears to be

different from the interferon response that results from dsRNA-mediated activation of protein kinase PKR and 2', 5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L.

The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as Dicer. Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs) (Bernstein *et al.*, 2001, *Nature*, 409, 363). Short interfering RNAs derived from Dicer activity are typically about 21 to about 23 nucleotides in length and comprise about 19 base pair duplexes. Dicer has also been implicated in the excision of 21- and 22-nucleotide small temporal RNAs (stRNAs) from precursor RNA of conserved structure that are implicated in translational control (Hutvagner *et al.*, 2001, *Science*, 293, 834). The RNAi response also features an endonuclease complex containing a siRNA, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded RNA having sequence homologous to the siRNA. Cleavage of the target RNA takes place in the middle of the region complementary to the guide sequence of the siRNA duplex (Elbashir *et al.*, 2001, *Genes Dev.*, 15, 188). In addition, RNA interference can also involve small RNA (e.g., micro-RNA or miRNA) mediated gene silencing, presumably through cellular mechanisms that regulate chromatin structure and thereby prevent transcription of target gene sequences (see for example Allshire, 2002, *Science*, 297, 1818-1819; Volpe *et al.*, 2002, *Science*, 297, 1833-1837; Jenuwein, 2002, *Science*, 297, 2215-2218; and Hall *et al.*, 2002, *Science*, 297, 2232-2237). As such, siRNA molecules of the invention can be used to mediate gene silencing via interaction with RNA transcripts or alternately by interaction with particular gene sequences, wherein such interaction results in gene silencing either at the transcriptional level or post-transcriptional level.

RNAi has been studied in a variety of systems. Fire *et al.*, 1998, *Nature*, 391, 806, were the first to observe RNAi in *C. elegans*. Wianny and Goetz, 1999, *Nature Cell Biol.*, 2, 70, describe RNAi mediated by dsRNA in mouse embryos. Hammond *et al.*, 2000, *Nature*, 404, 293, describe RNAi in *Drosophila* cells transfected with dsRNA. Elbashir *et al.*, 2001, *Nature*, 411, 494, describe RNAi induced by introduction of duplexes of synthetic 21-nucleotide RNAs in cultured mammalian cells including human

embryonic kidney and HeLa cells. Recent work in *Drosophila* embryonic lysates has revealed certain requirements for siRNA length, structure, chemical composition, and sequence that are essential to mediate efficient RNAi activity. These studies have shown that 21 nucleotide siRNA duplexes are most active when containing two 2-nucleotide 3'-terminal nucleotide overhangs. Furthermore, substitution of one or both siRNA strands with 2'-deoxy or 2'-O-methyl nucleotides abolishes RNAi activity, whereas substitution of 3'-terminal siRNA nucleotides with deoxy nucleotides was shown to be tolerated. Mismatch sequences in the center of the siRNA duplex were also shown to abolish RNAi activity. In addition, these studies also indicate that the position of the cleavage site in the target RNA is defined by the 5'-end of the siRNA guide sequence rather than the 3'-end (Elbashir *et al.*, 2001, *EMBO J.*, 20, 6877). Other studies have indicated that a 5'-phosphate on the target-complementary strand of a siRNA duplex is required for siRNA activity and that ATP is utilized to maintain the 5'-phosphate moiety on the siRNA (Nykanen *et al.*, 2001, *Cell*, 107, 309); however, siRNA molecules lacking a 5'-phosphate are active when introduced exogenously, suggesting that 5'-phosphorylation of siRNA constructs may occur *in vivo*.

Synthesis of Nucleic Acid Molecules

Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this invention, small nucleic acid motifs ("small" refers to nucleic acid motifs no more than 100 nucleotides in length, preferably no more than 80 nucleotides in length, and most preferably no more than 50 nucleotides in length; *e.g.*, individual siNA oligonucleotide sequences or siNA sequences synthesized in tandem) are preferably used for exogenous delivery. The simple structure of these molecules increases the ability of the nucleic acid to invade targeted regions of protein and/or RNA structure. Exemplary molecules of the instant invention are chemically synthesized, and others can similarly be synthesized.

Oligonucleotides (*e.g.*, certain modified oligonucleotides or portions of oligonucleotides lacking ribonucleotides) are synthesized using protocols known in the art, for example as described in Caruthers *et al.*, 1992, *Methods in Enzymology* 211, 3-19, Thompson *et al.*, International PCT Publication No. WO 99/54459, Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677-2684, Wincott *et al.*, 1997, *Methods Mol. Bio.*, 74,

59, Brennan *et al.*, 1998, *Biotechnol Bioeng.*, 61, 33-45, and Brennan, U.S. Pat. No. 6,001,311. All of these references are incorporated herein by reference. The synthesis of oligonucleotides makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2 μmol scale protocol with a 2.5 min coupling step for 2'-O-methylated nucleotides and a 45 second coupling step for 2'-deoxy nucleotides or 2'-deoxy-2'-fluoro nucleotides. Table V outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 μmol scale can be performed on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) with minimal modification to the cycle. A 33-fold excess (60 μL of 0.11 M = 6.6 μmol) of 2'-O-methyl phosphoramidite and a 105-fold excess of S-ethyl tetrazole (60 μL of 0.25 M = 15 μmol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 22-fold excess (40 μL of 0.11 M = 4.4 μmol) of deoxy phosphoramidite and a 70-fold excess of S-ethyl tetrazole (40 μL of 0.25 M = 10 μmol) can be used in each coupling cycle of deoxy residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include the following: detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% *N*-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); and oxidation solution is 16.9 mM I_2 , 49 mM pyridine, 9% water in THF (PerSeptive Biosystems, Inc.). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide, 0.05 M in acetonitrile) is used.

Deprotection of the DNA-based oligonucleotides is performed as follows: the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aqueous methylamine (1 mL) at 65 °C for 10 minutes. After cooling to -20 °C, the supernatant is removed from the polymer support.

The support is washed three times with 1.0 mL of EtOH:MeCN:H₂O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder.

The method of synthesis used for RNA including certain siNA molecules of the invention follows the procedure as described in Usman *et al.*, 1987, *J. Am. Chem. Soc.*, 109, 7845; Scaringe *et al.*, 1990, *Nucleic Acids Res.*, 18, 5433; and Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677-2684 Wincott *et al.*, 1997, *Methods Mol. Bio.*, 74, 59, and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2 μ mol scale protocol with a 7.5 min coupling step for alkylsilyl protected nucleotides and a 2.5 min coupling step for 2'-O-methylated nucleotides. Table V outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 μ mol scale can be done on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) with minimal modification to the cycle. A 33-fold excess (60 μ L of 0.11 M = 6.6 μ mol) of 2'-O-methyl phosphoramidite and a 75-fold excess of S-ethyl tetrazole (60 μ L of 0.25 M = 15 μ mol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 66-fold excess (120 μ L of 0.11 M = 13.2 μ mol) of alkylsilyl (ribo) protected phosphoramidite and a 150-fold excess of S-ethyl tetrazole (120 μ L of 0.25 M = 30 μ mol) can be used in each coupling cycle of ribo residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include the following: detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% *N*-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); oxidation solution is 16.9 mM I₂, 49 mM pyridine, 9% water in THF (PerSeptive Biosystems, Inc.). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate

linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide 0.05 M in acetonitrile) is used.

Deprotection of the RNA is performed using either a two-pot or one-pot protocol. For the two-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aq. methylamine (1 mL) at 65 °C for 10 min. After cooling to -20 °C, the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H₂O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder. The base deprotected oligoribonucleotide is resuspended in anhydrous TEA/HF/NMP solution (300 µL of a solution of 1.5 mL N-methylpyrrolidinone, 750 µL TEA and 1 mL TEA•3HF to provide a 1.4 M HF concentration) and heated to 65 °C. After 1.5 h, the oligomer is quenched with 1.5 M NH₄HCO₃.

Alternatively, for the one-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 33% ethanolic methylamine/DMSO: 1/1 (0.8 mL) at 65 °C for 15 minutes. The vial is brought to room temperature TEA•3HF (0.1 mL) is added and the vial is heated at 65 °C for 15 minutes. The sample is cooled at -20 °C and then quenched with 1.5 M NH₄HCO₃.

For purification of the trityl-on oligomers, the quenched NH₄HCO₃ solution is loaded onto a C-18 containing cartridge that had been prewashed with acetonitrile followed by 50 mM TEAA. After washing the loaded cartridge with water, the RNA is detritylated with 0.5% TFA for 13 minutes. The cartridge is then washed again with water, salt exchanged with 1 M NaCl and washed with water again. The oligonucleotide is then eluted with 30% acetonitrile.

The average stepwise coupling yields are typically >98% (Wincott *et al.*, 1995 *Nucleic Acids Res.* 23, 2677-2684). Those of ordinary skill in the art will recognize that the scale of synthesis can be adapted to be larger or smaller than the example described above including but not limited to 96-well format.

Alternatively, the nucleic acid molecules of the present invention can be synthesized separately and joined together post-synthetically, for example, by ligation (Moore *et al.*, 1992, *Science* 256, 9923; Draper *et al.*, International PCT publication No. WO 93/23569; Shabarova *et al.*, 1991, *Nucleic Acids Research* 19, 4247; Bellon *et al.*, 5 1997, *Nucleosides & Nucleotides*, 16, 951; Bellon *et al.*, 1997, *Bioconjugate Chem.* 8, 204), or by hybridization following synthesis and/or deprotection.

The siNA molecules of the invention can also be synthesized via a tandem synthesis methodology as described in Example 1 herein, wherein both siNA strands are synthesized as a single contiguous oligonucleotide fragment or strand separated by a 10 cleavable linker which is subsequently cleaved to provide separate siNA fragments or strands that hybridize and permit purification of the siNA duplex. The linker can be a polynucleotide linker or a non-nucleotide linker. The tandem synthesis of siNA as described herein can be readily adapted to both multiwell/multiplate synthesis platforms such as 96 well or similarly larger multi-well platforms. The tandem synthesis of siNA as 15 described herein can also be readily adapted to large scale synthesis platforms employing batch reactors, synthesis columns and the like.

A siNA molecule can also be assembled from two distinct nucleic acid strands or fragments wherein one fragment includes the sense region and the second fragment includes the antisense region of the RNA molecule.

20 The nucleic acid molecules of the present invention can be modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 2'-O-methyl, 2'-H (for a review see Usman and Cedergren, 1992, *TIBS* 17, 34; Usman *et al.*, 1994, *Nucleic Acids Symp. Ser.* 31, 163). siNA constructs can be purified by gel electrophoresis using general methods or can be purified by high 25 pressure liquid chromatography (HPLC; see Wincott *et al.*, *supra*, the totality of which is hereby incorporated herein by reference) and re-suspended in water.

In another aspect of the invention, siNA molecules of the invention are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed 30 based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus.

The recombinant vectors capable of expressing the siNA molecules can be delivered as described herein, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of siNA molecules.

Optimizing Activity of the nucleic acid molecule of the invention.

- 5 Chemically synthesizing nucleic acid molecules with modifications (base, sugar and/or phosphate) can prevent their degradation by serum ribonucleases, which can increase their potency (see *e.g.*, Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*, 1990 *Nature* 344, 565; Pieken *et al.*, 1991, *Science* 253, 314; Usman and Cedergren, 1992, *Trends in Biochem. Sci.* 17, 334; Usman *et al.*,
10 International Publication No. WO 93/15187; and Rossi *et al.*, International Publication No. WO 91/03162; Sproat, U.S. Pat. No. 5,334,711; Gold *et al.*, U.S. Pat. No. 6,300,074; and Burgin *et al.*, *supra*; all of which are incorporated by reference herein). All of the above references describe various chemical modifications that can be made to the base, phosphate and/or sugar moieties of the nucleic acid molecules described herein.
15 Modifications that enhance their efficacy in cells, and removal of bases from nucleic acid molecules to shorten oligonucleotide synthesis times and reduce chemical requirements are desired.

- There are several examples in the art describing sugar, base and phosphate modifications that can be introduced into nucleic acid molecules with significant
20 enhancement in their nuclease stability and efficacy. For example, oligonucleotides are modified to enhance stability and/or enhance biological activity by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 2'-O-methyl, 2'-O-allyl, 2'-H, nucleotide base modifications (for a review see Usman and Cedergren, 1992, *TIBS*, 17, 34; Usman *et al.*, 1994, *Nucleic Acids Symp. Ser.* 31, 163; Burgin *et al.*, 1996,
25 *Biochemistry*, 35, 14090). Sugar modification of nucleic acid molecules have been extensively described in the art (see Eckstein *et al.*, International Publication PCT No. WO 92/07065; Perrault *et al.*, *Nature*, 1990, 344, 565-568; Pieken *et al.*, *Science*, 1991, 253, 314-317; Usman and Cedergren, *Trends in Biochem. Sci.*, 1992, 17, 334-339; Usman *et al.*, International Publication PCT No. WO 93/15187; Sproat, U.S. Pat. No.
30 5,334,711 and Beigelman *et al.*, 1995, *J. Biol. Chem.*, 270, 25702; Beigelman *et al.*, International PCT publication No. WO 97/26270; Beigelman *et al.*, U.S. Pat. No.

5,716,824; Usman *et al.*, U.S. Pat. No. 5,627,053; Woolf *et al.*, International PCT Publication No. WO 98/13526; Thompson *et al.*, USSN 60/082,404 which was filed on April 20, 1998; Karpeisky *et al.*, 1998, *Tetrahedron Lett.*, 39, 1131; Earnshaw and Gait, 1998, *Biopolymers (Nucleic Acid Sciences)*, 48, 39-55; Verma and Eckstein, 1998, *Annu. Rev. Biochem.*, 67, 99-134; and Burlina *et al.*, 1997, *Bioorg. Med. Chem.*, 5, 1999-2010; all of the references are hereby incorporated in their totality by reference herein). Such publications describe general methods and strategies to determine the location of incorporation of sugar, base and/or phosphate modifications and the like into nucleic acid molecules without modulating catalysis, and are incorporated by reference herein. In view of such teachings, similar modifications can be used as described herein to modify the siNA nucleic acid molecules of the instant invention so long as the ability of siNA to promote RNAi in cells is not significantly inhibited.

While chemical modification of oligonucleotide internucleotide linkages with phosphorothioate, phosphorodithioate, and/or 5'-methylphosphonate linkages improves stability, excessive modifications can cause some toxicity or decreased activity. Therefore, when designing nucleic acid molecules, the amount of these internucleotide linkages should be minimized. The reduction in the concentration of these linkages should lower toxicity, resulting in increased efficacy and higher specificity of these molecules.

Short interfering nucleic acid (siNA) molecules having chemical modifications that maintain or enhance activity are provided. Such a nucleic acid is also generally more resistant to nucleases than an unmodified nucleic acid. Accordingly, the *in vitro* and/or *in vivo* activity should not be significantly lowered. In cases in which modulation is the goal, therapeutic nucleic acid molecules delivered exogenously should optimally be stable within cells until translation of the target RNA has been modulated long enough to reduce the levels of the undesirable protein. This period of time varies between hours to days depending upon the disease state. Improvements in the chemical synthesis of RNA and DNA (Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677; Caruthers *et al.*, 1992, *Methods in Enzymology* 211, 3-19 (incorporated by reference herein)) have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability, as described above.

In one embodiment, nucleic acid molecules of the invention include one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) G-clamp nucleotides. A G-clamp nucleotide is a modified cytosine analog wherein the modifications confer the ability to hydrogen bond both Watson-Crick and Hoogsteen faces of a complementary guanine within a duplex, see for example Lin and Matteucci, 1998, *J. Am. Chem. Soc.*, 120, 8531-8532. A single G-clamp analog substitution within an oligonucleotide can result in substantially enhanced helical thermal stability and mismatch discrimination when hybridized to complementary oligonucleotides. The inclusion of such nucleotides in nucleic acid molecules of the invention results in both enhanced affinity and specificity to nucleic acid targets, complementary sequences, or template strands. In another embodiment, nucleic acid molecules of the invention include one or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) LNA "locked nucleic acid" nucleotides such as a 2', 4'-C methylene bicyclo nucleotide (see for example Wengel *et al.*, International PCT Publication No. WO 00/66604 and WO 99/14226).

In another embodiment, the invention features conjugates and/or complexes of siNA molecules of the invention. Such conjugates and/or complexes can be used to facilitate delivery of siNA molecules into a biological system, such as a cell. The conjugates and complexes provided by the instant invention can impart therapeutic activity by transferring therapeutic compounds across cellular membranes, altering the pharmacokinetics, and/or modulating the localization of nucleic acid molecules of the invention. The present invention encompasses the design and synthesis of novel conjugates and complexes for the delivery of molecules, including, but not limited to, small molecules, lipids, cholesterol, phospholipids, nucleosides, nucleotides, nucleic acids, antibodies, toxins, negatively charged polymers and other polymers, for example proteins, peptides, hormones, carbohydrates, polyethylene glycols, or polyamines, across cellular membranes. In general, the transporters described are designed to be used either individually or as part of a multi-component system, with or without degradable linkers. These compounds are expected to improve delivery and/or localization of nucleic acid molecules of the invention into a number of cell types originating from different tissues, in the presence or absence of serum (see Sullenger and Cech, U.S. Pat. No. 5,854,038). Conjugates of the molecules described herein can be attached to biologically active

molecules via linkers that are biodegradable, such as biodegradable nucleic acid linker molecules.

The term "biodegradable linker" as used herein, refers to a nucleic acid or non-nucleic acid linker molecule that is designed as a biodegradable linker to connect one molecule to another molecule, for example, a biologically active molecule to a siNA molecule of the invention or the sense and antisense strands of a siNA molecule of the invention. The biodegradable linker is designed such that its stability can be modulated for a particular purpose, such as delivery to a particular tissue or cell type. The stability of a nucleic acid-based biodegradable linker molecule can be modulated by using various chemistries, for example combinations of ribonucleotides, deoxyribonucleotides, and chemically-modified nucleotides, such as 2'-O-methyl, 2'-fluoro, 2'-amino, 2'-O-amino, 2'-C-allyl, 2'-O-allyl, and other 2'-modified or base modified nucleotides. The biodegradable nucleic acid linker molecule can be a dimer, trimer, tetramer or longer nucleic acid molecule, for example, an oligonucleotide of about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides in length, or can comprise a single nucleotide with a phosphorus-based linkage, for example, a phosphoramidate or phosphodiester linkage. The biodegradable nucleic acid linker molecule can also comprise nucleic acid backbone, nucleic acid sugar, or nucleic acid base modifications.

The term "biodegradable" as used herein, refers to degradation in a biological system, for example enzymatic degradation or chemical degradation.

The term "biologically active molecule" as used herein, refers to compounds or molecules that are capable of eliciting or modifying a biological response in a system. Non-limiting examples of biologically active siNA molecules either alone or in combination with other molecules contemplated by the instant invention include therapeutically active molecules such as antibodies, cholesterol, hormones, antivirals, peptides, proteins, chemotherapeutics, small molecules, vitamins, co-factors, nucleosides, nucleotides, oligonucleotides, enzymatic nucleic acids, antisense nucleic acids, triplex forming oligonucleotides, 2,5-A chimeras, siNA, dsRNA, allozymes, aptamers, decoys and analogs thereof. Biologically active molecules of the invention also include molecules capable of modulating the pharmacokinetics and/or

pharmacodynamics of other biologically active molecules, for example, lipids and polymers such as polyamines, polyamides, polyethylene glycol and other polyethers.

The term "phospholipid" as used herein, refers to a hydrophobic molecule comprising at least one phosphorus group. For example, a phospholipid can comprise a phosphorus-containing group and saturated or unsaturated alkyl group, optionally substituted with OH, COOH, oxo, amine, or substituted or unsubstituted aryl groups.

Therapeutic nucleic acid molecules (*e.g.*, siNA molecules) delivered exogenously optimally are stable within cells until reverse transcription of the RNA has been modulated long enough to reduce the levels of the RNA transcript. The nucleic acid molecules are resistant to nucleases in order to function as effective intracellular therapeutic agents. Improvements in the chemical synthesis of nucleic acid molecules described in the instant invention and in the art have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability as described above.

In yet another embodiment, siNA molecules having chemical modifications that maintain or enhance enzymatic activity of proteins involved in RNAi are provided. Such nucleic acids are also generally more resistant to nucleases than unmodified nucleic acids. Thus, *in vitro* and/or *in vivo* the activity should not be significantly lowered.

Use of the nucleic acid-based molecules of the invention will lead to better treatments by affording the possibility of combination therapies (*e.g.*, multiple siNA molecules targeted to different genes; nucleic acid molecules coupled with known small molecule modulators; or intermittent treatment with combinations of molecules, including different motifs and/or other chemical or biological molecules). The treatment of subjects with siNA molecules can also include combinations of different types of nucleic acid molecules, such as enzymatic nucleic acid molecules (ribozymes), allozymes, antisense, 2,5-A oligoadenylate, decoys, and aptamers.

In another aspect a siNA molecule of the invention comprises one or more 5' and/or a 3'- cap structure, for example on only the sense siNA strand, the antisense siNA strand, or both siNA strands.

By "cap structure" is meant chemical modifications, which have been incorporated at either terminus of the oligonucleotide (see, for example, Adamic *et al.*, U.S. Pat. No. 5,998,203, incorporated by reference herein). These terminal modifications protect the nucleic acid molecule from exonuclease degradation, and may help in delivery and/or localization within a cell. The cap may be present at the 5'-terminus (5'-cap) or at the 3'-terminal (3'-cap) or may be present on both termini. In non-limiting examples, the 5'-cap includes, but is not limited to, glyceryl, inverted deoxy abasic residue (moiety); 4',5'-methylene nucleotide; 1-(beta-D-erythrofuransyl) nucleotide, 4'-thio nucleotide; carbocyclic nucleotide; 1,5-anhydrohexitol nucleotide; L-nucleotides; alpha-nucleotides; modified base nucleotide; phosphorodithioate linkage; *threo*-pentofuransyl nucleotide; acyclic 3',4'-seco nucleotide; acyclic 3,4-dihydroxybutyl nucleotide; acyclic 3,5-dihydroxypentyl nucleotide, 3'-3'-inverted nucleotide moiety; 3'-3'-inverted abasic moiety; 3'-2'-inverted nucleotide moiety; 3'-2'-inverted abasic moiety; 1,4-butanediol phosphate; 3'-phosphoramidate; hexylphosphate; aminohexyl phosphate; 3'-phosphate; 3'-phosphorothioate; phosphorodithioate; or bridging or non-bridging methylphosphonate moiety.

Non-limiting examples of the 3'-cap include, but are not limited to, glyceryl, inverted deoxy abasic residue (moiety), 4', 5'-methylene nucleotide; 1-(beta-D-erythrofuransyl) nucleotide; 4'-thio nucleotide, carbocyclic nucleotide; 5'-amino-alkyl phosphate; 1,3-diamino-2-propyl phosphate; 3-aminopropyl phosphate; 6-aminohexyl phosphate; 1,2-aminododecyl phosphate; hydroxypropyl phosphate; 1,5-anhydrohexitol nucleotide; L-nucleotide; alpha-nucleotide; modified base nucleotide; phosphorodithioate; *threo*-pentofuransyl nucleotide; acyclic 3',4'-seco nucleotide; 3,4-dihydroxybutyl nucleotide; 3,5-dihydroxypentyl nucleotide, 5'-5'-inverted nucleotide moiety; 5'-5'-inverted abasic moiety; 5'-phosphoramidate; 5'-phosphorothioate; 1,4-butanediol phosphate; 5'-amino; bridging and/or non-bridging 5'-phosphoramidate, phosphorothioate and/or phosphorodithioate, bridging or non bridging methylphosphonate and 5'-mercapto moieties (for more details see Beaucage and Iyer, 1993, *Tetrahedron* 49, 1925; incorporated by reference herein).

By the term "non-nucleotide" is meant any group or compound which can be incorporated into a nucleic acid chain in the place of one or more nucleotide units,

including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound is abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine and therefore lacks a base at the 1'-position.

5 An "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably, it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group can be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂,
10 amino, or SH. The term also includes alkenyl groups that are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably, it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the
15 substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂, halogen, N(CH₃)₂, amino, or SH. The term "alkyl" also includes alkynyl groups that have an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably, it is a lower alkynyl of from 1 to 7 carbons,
20 more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino or SH.

Such alkyl groups can also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group that has at
25 least one ring having a conjugated pi electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as described
30 above). Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl

groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An

5 "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

By "nucleotide" as used herein is as recognized in the art to include natural bases (standard), and modified bases well known in the art. Such bases are generally located at the 1' position of a nucleotide sugar moiety. Nucleotides generally comprise a base,

10 sugar and a phosphate group. The nucleotides can be unmodified or modified at the sugar, phosphate and/or base moiety, (also referred to interchangeably as nucleotide analogs, modified nucleotides, non-natural nucleotides, non-standard nucleotides and other; see, for example, Usman and McSwiggen, *supra*; Eckstein *et al.*, International PCT Publication No. WO 92/07065; Usman *et al.*, International PCT Publication No.

15 WO 93/15187; Uhlman & Peyman, *supra*, all are hereby incorporated by reference herein). There are several examples of modified nucleic acid bases known in the art as summarized by Limbach *et al.*, 1994, *Nucleic Acids Res.* 22, 2183. Some of the non-limiting examples of base modifications that can be introduced into nucleic acid molecules include, inosine, purine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2,

20 4, 6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidines (*e.g.*, 5-methylcytidine), 5-alkyluridines (*e.g.*, ribothymidine), 5-halouridine (*e.g.*, 5-bromouridine) or 6-azapyrimidines or 6-alkylpyrimidines (*e.g.* 6-methyluridine), propyne, and others (Burgin *et al.*, 1996, *Biochemistry*, 35, 14090; Uhlman & Peyman, *supra*). By "modified bases" in this aspect is meant nucleotide bases

25 other than adenine, guanine, cytosine and uracil at 1' position or their equivalents.

In one embodiment, the invention features modified siNA molecules, with phosphate backbone modifications comprising one or more phosphorothioate, phosphorodithioate, methylphosphonate, phosphotriester, morpholino, amidate carbamate, carboxymethyl, acetamidate, polyamide, sulfonate, sulfonamide, sulfamate,

30 formacetal, thioformacetal, and/or alkylsilyl, substitutions. For a review of oligonucleotide backbone modifications, see Hunziker and Leumann, 1995, *Nucleic Acid*

Analogues: Synthesis and Properties, in *Modern Synthetic Methods*, VCH, 331-417, and Mesmaeker *et al.*, 1994, *Novel Backbone Replacements for Oligonucleotides*, in *Carbohydrate Modifications in Antisense Research*, ACS, 24-39.

By "abasic" is meant sugar moieties lacking a base or having other chemical groups in place of a base at the 1' position, see for example Adamic *et al.*, U.S. Pat. No. 5,998,203.

By "unmodified nucleoside" is meant one of the bases adenine, cytosine, guanine, thymine, or uracil joined to the 1' carbon of β -D-ribo-furanose.

By "modified nucleoside" is meant any nucleotide base which contains a modification in the chemical structure of an unmodified nucleotide base, sugar and/or phosphate. Non-limiting examples of modified nucleotides are shown by Formulae I-VII and/or other modifications described herein.

In connection with 2'-modified nucleotides as described for the present invention, by "amino" is meant 2'-NH₂ or 2'-O-NH₂, which can be modified or unmodified. Such modified groups are described, for example, in Eckstein *et al.*, U.S. Pat. No. 5,672,695 and Matulic-Adamic *et al.*, U.S. Pat. No. 6,248,878, which are both incorporated by reference in their entireties.

Various modifications to nucleic acid siNA structure can be made to enhance the utility of these molecules. Such modifications will enhance shelf-life, half-life *in vitro*, stability, and ease of introduction of such oligonucleotides to the target site, *e.g.*, to enhance penetration of cellular membranes, and confer the ability to recognize and bind to targeted cells.

Administration of Nucleic Acid Molecules

A siNA molecule of the invention can be adapted for use to treat, for example, neurodegenerative diseases, disorders, or conditions such as Parkinson's disease, Alzheimer's disease, dementia, and any other diseases or conditions that are related to or will respond to the levels of PARK1, PARK2, PARK5, and/or PARK7 in a cell or tissue, alone or in combination with other therapies. For example, a siNA molecule can

comprise a delivery vehicle, including liposomes, for administration to a subject, carriers and diluents and their salts, and/or can be present in pharmaceutically acceptable formulations. Methods for the delivery of nucleic acid molecules are described in Akhtar *et al.*, 1992, *Trends Cell Bio.*, 2, 139; *Delivery Strategies for Antisense*
5 *Oligonucleotide Therapeutics*, ed. Akhtar, 1995, Maurer *et al.*, 1999, *Mol. Membr. Biol.*, 16, 129-140; Hofland and Huang, 1999, *Handb. Exp. Pharmacol.*, 137, 165-192; and Lee *et al.*, 2000, *ACS Symp. Ser.*, 752, 184-192, all of which are incorporated herein by reference. Beigelman *et al.*, U.S. Pat. No. 6,395,713 and Sullivan *et al.*, PCT WO 94/02595 further describe the general methods for delivery of nucleic acid molecules.
10 These protocols can be utilized for the delivery of virtually any nucleic acid molecule. Many examples in the art describe CNS delivery methods of oligonucleotides by osmotic pump, (see Chun *et al.*, 1998, *Neuroscience Letters*, 257, 135-138, D'Aldin *et al.*, 1998, *Mol. Brain Research*, 55, 151-164, Dryden *et al.*, 1998, *J. Endocrinol.*, 157, 169-175, Ghirmikar *et al.*, 1998, *Neuroscience Letters*, 247, 21-24) or direct infusion (Broadus *et al.*, 1997, *Neurosurg. Focus*, 3, article 4). Other routes of delivery include, but are not
15 limited to oral (tablet or pill form) and/or intrathecal delivery (Gold, 1997, *Neuroscience*, 76, 1153-1158). For a comprehensive review on drug delivery strategies including broad coverage of CNS delivery, see Ho *et al.*, 1999, *Curr. Opin. Mol. Ther.*, 1, 336-343 and Jain, *Drug Delivery Systems: Technologies and Commercial Opportunities*, Decision
20 Resources, 1998 and Groothuis *et al.*, 1997, *J. NeuroVirol.*, 3, 387-400. Nucleic acid molecules can be administered to cells by a variety of methods known to those of skill in the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as biodegradable polymers, hydrogels, cyclodextrins (see for example Gonzalez *et al.*, 1999, *Bioconjugate Chem.*, 10, 1068-
25 1074; Wang *et al.*, International PCT publication Nos. WO 03/47518 and WO 03/46185), poly(lactic-co-glycolic)acid (PLGA) and PLGA microspheres (see for example US Patent 6,447,796 and US Patent Application Publication No. US 2002130430), biodegradable nanocapsules, and bioadhesive microspheres, or by proteinaceous vectors (O'Hare and Normand, International PCT Publication No. WO
30 00/53722). Alternatively, the nucleic acid/vehicle combination is locally delivered by direct injection or by use of an infusion pump. Direct injection of the nucleic acid molecules of the invention, whether subcutaneous, intramuscular, or intradermal, can

take place using standard needle and syringe methodologies, or by needle-free technologies such as those described in Conry *et al.*, 1999, *Clin. Cancer Res.*, 5, 2330-2337 and Barry *et al.*, International PCT Publication No. WO 99/31262. The molecules of the instant invention can be used as pharmaceutical agents. Pharmaceutical agents

5 prevent, modulate the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state in a subject.

These protocols can be utilized for the delivery of virtually any nucleic acid molecule. Nucleic acid molecules can be administered to cells by a variety of methods known to those of skill in the art, including, but not restricted to, encapsulation in

10 liposomes, by iontophoresis, or by incorporation into other vehicles, such as biodegradable polymers, hydrogels, cyclodextrins (see for example Gonzalez *et al.*, 1999, *Bioconjugate Chem.*, 10, 1068-1074; Wang *et al.*, International PCT publication Nos. WO 03/47518 and WO 03/46185), poly(lactic-co-glycolic)acid (PLGA) and PLGA microspheres (see for example US Patent 6,447,796 and US Patent Application

15 Publication No. US 2002130430), biodegradable nanocapsules, and bioadhesive microspheres, or by proteinaceous vectors (O'Hare and Normand, International PCT Publication No. WO 00/53722). In another embodiment, the nucleic acid molecules of the invention can also be formulated or complexed with polyethyleneimine and derivatives thereof, such as polyethyleneimine-polyethyleneglycol-N-

20 acetylgalactosamine (PEI-PEG-GAL) or polyethyleneimine-polyethyleneglycol-tri-N-acetylgalactosamine (PEI-PEG-triGAL) derivatives.

Experiments have demonstrated the efficient *in vivo* uptake of nucleic acids by neurons. As an example of local administration of nucleic acids to nerve cells, Sommer *et al.*, 1998, *Antisense Nuc. Acid Drug Dev.*, 8, 75, describe a study in which a 15mer

25 phosphorothioate antisense nucleic acid molecule to c-fos is administered to rats via microinjection into the brain. Antisense molecules labeled with tetramethylrhodamine-isothiocyanate (TRITC) or fluorescein isothiocyanate (FITC) were taken up by exclusively by neurons thirty minutes post-injection. A diffuse cytoplasmic staining and nuclear staining was observed in these cells. As an example of systemic administration

30 of nucleic acid to nerve cells, Epa *et al.*, 2000, *Antisense Nuc. Acid Drug Dev.*, 10, 469, describe an *in vivo* mouse study in which beta-cyclodextrin-adamantane-oligonucleotide

conjugates were used to target the p75 neurotrophin receptor in neuronally differentiated PC12 cells. Following a two week course of IP administration, pronounced uptake of p75 neurotrophin receptor antisense was observed in dorsal root ganglion (DRG) cells. In addition, a marked and consistent down-regulation of p75 was observed in DRG
5 neurons. Additional approaches to the targeting of nucleic acid to neurons are described in Broaddus *et al.*, 1998, *J. Neurosurg.*, 88(4), 734; Karle *et al.*, 1997, *Eur. J. Pharmacol.*, 340(2/3), 153; Bannai *et al.*, 1998, *Brain Research*, 784(1,2), 304; Rajakumar *et al.*, 1997, *Synapse*, 26(3), 199; Wu-pong *et al.*, 1999, *BioPharm*, 12(1), 32; Bannai *et al.*, 1998, *Brain Res. Protoc.*, 3(1), 83; Simantov *et al.*, 1996, *Neuroscience*,
10 74(1), 39. Nucleic acid molecules of the invention are therefore amenable to delivery to and uptake by cells that express PARK1, PARK2, PARK5, and/or PARK7 for modulation of PARK1, PARK2, PARK5, and/or PARK7 expression.

The delivery of nucleic acid molecules of the invention, targeting PARK1, PARK2, PARK5, and/or PARK7 is provided by a variety of different strategies.
15 Traditional approaches to CNS delivery that can be used include, but are not limited to, intrathecal and intracerebroventricular administration, implantation of catheters and pumps, direct injection or perfusion at the site of injury or lesion, injection into the brain arterial system, or by chemical or osmotic opening of the blood-brain barrier. Other approaches can include the use of various transport and carrier systems, for example
20 though the use of conjugates and biodegradable polymers. Furthermore, gene therapy approaches, for example as described in Kaplitt *et al.*, US 6,180,613, can be used to express nucleic acid molecules in the CNS.

In one embodiment, a siNA molecule of the invention is complexed with membrane disruptive agents such as those described in U.S. Patent Application
25 Publication No. 20010007666, incorporated by reference herein in its entirety including the drawings. In another embodiment, the membrane disruptive agent or agents and the siNA molecule are also complexed with a cationic lipid or helper lipid molecule, such as those lipids described in U.S. Patent No. 6,235,310, incorporated by reference herein in its entirety including the drawings.

30 In one embodiment, siNA molecules of the invention are formulated or complexed with polyethylenimine (e.g., linear or branched PEI) and/or polyethylenimine derivatives, including for example grafted PEIs such as galactose PEI, cholesterol PEI,

antibody derivatized PEI, and polyethylene glycol PEI (PEG-PEI) derivatives thereof (see for example Ogris *et al.*, 2001, *AAPA PharmSci*, 3, 1-11; Furgeson *et al.*, 2003, *Bioconjugate Chem.*, 14, 840-847; Kunath *et al.*, 2002, *Pharmaceutical Research*, 19, 810-817; Choi *et al.*, 2001, *Bull. Korean Chem. Soc.*, 22, 46-52; Bettinger *et al.*, 1999, 5 *Bioconjugate Chem.*, 10, 558-561; Peterson *et al.*, 2002, *Bioconjugate Chem.*, 13, 845-854; Erbacher *et al.*, 1999, *Journal of Gene Medicine Preprint*, 1, 1-18; Godbey *et al.*, 1999, *PNAS USA*, 96, 5177-5181; Godbey *et al.*, 1999, *Journal of Controlled Release*, 60, 149-160; Diebold *et al.*, 1999, *Journal of Biological Chemistry*, 274, 19087-19094; Thomas and Klibanov, 2002, *PNAS USA*, 99, 14640-14645; and Sagara, US 6,586,524, 10 incorporated by reference herein.

In one embodiment, a siNA molecule of the invention is complexed with membrane disruptive agents such as those described in U.S. Patent Application Publication No. 20010007666, incorporated by reference herein in its entirety including the drawings. In another embodiment, the membrane disruptive agent or agents and the 15 siNA molecule are also complexed with a cationic lipid or helper lipid molecule, such as those lipids described in U.S. Patent No. 6,235,310, incorporated by reference herein in its entirety including the drawings.

In one embodiment, a siNA molecule of the invention is complexed with delivery systems as described in U.S. Patent Application Publication No. 2003077829 and 20 International PCT Publication Nos. WO 00/03683 and WO 02/087541, all incorporated by reference herein in their entirety including the drawings.

Thus, the invention features a pharmaceutical composition comprising one or more nucleic acid(s) of the invention in an acceptable carrier, such as a stabilizer, buffer, and the like. The polynucleotides of the invention can be administered (*e.g.*, RNA, DNA or 25 protein) and introduced to a subject by any standard means, with or without stabilizers, buffers, and the like, to form a pharmaceutical composition. When it is desired to use a liposome delivery mechanism, standard protocols for formation of liposomes can be followed. The compositions of the present invention can also be formulated and used as creams, gels, sprays, oils and other suitable compositions for topical, dermal, or 30 transdermal administration as is known in the art.

The present invention also includes pharmaceutically acceptable formulations of the compounds described. These formulations include salts of the above compounds, *e.g.*, acid addition salts, for example, salts of hydrochloric, hydrobromic, acetic acid, and benzene sulfonic acid.

5 A pharmacological composition or formulation refers to a composition or formulation in a form suitable for administration, *e.g.*, systemic or local administration, into a cell or subject, including for example a human. Suitable forms, in part, depend upon the use or the route of entry, for example oral, transdermal, or by injection. Such forms should not prevent the composition or formulation from reaching a target cell (*i.e.*,
10 a cell to which the negatively charged nucleic acid is desirable for delivery). For example, pharmacological compositions injected into the blood stream should be soluble. Other factors are known in the art, and include considerations such as toxicity and forms that prevent the composition or formulation from exerting its effect.

By "systemic administration" is meant *in vivo* systemic absorption or accumulation
15 of drugs in the blood stream followed by distribution throughout the entire body. Administration routes that lead to systemic absorption include, without limitation: intravenous, subcutaneous, intraperitoneal, inhalation, oral, intrapulmonary and intramuscular. Each of these administration routes exposes the siNA molecules of the invention to an accessible diseased tissue. The rate of entry of a drug into the
20 circulation has been shown to be a function of molecular weight or size. The use of a liposome or other drug carrier comprising the compounds of the instant invention can potentially localize the drug, for example, in certain tissue types, such as the tissues of the reticular endothelial system (RES). A liposome formulation that can facilitate the association of drug with the surface of cells, such as, lymphocytes and macrophages is
25 also useful. This approach can provide enhanced delivery of the drug to target cells by taking advantage of the specificity of macrophage and lymphocyte immune recognition of abnormal cells.

By "pharmaceutically acceptable formulation" is meant, a composition or formulation that allows for the effective distribution of the nucleic acid molecules of the
30 instant invention in the physical location most suitable for their desired activity. Non-limiting examples of agents suitable for formulation with the nucleic acid molecules of

the instant invention include: P-glycoprotein inhibitors (such as Pluronic P85); biodegradable polymers, such as poly (DL-lactide-coglycolide) microspheres for sustained release delivery (Emerich, DF *et al.*, 1999, *Cell Transplant.*, 8, 47-58); and loaded nanoparticles, such as those made of polybutylcyanoacrylate. Other non-limiting
5 examples of delivery strategies for the nucleic acid molecules of the instant invention include material described in Boado *et al.*, 1998, *J. Pharm. Sci.*, 87, 1308-1315; Tyler *et al.*, 1999, *FEBS Lett.*, 421, 280-284; Pardridge *et al.*, 1995, *PNAS USA.*, 92, 5592-5596; Boado, 1995, *Adv. Drug Delivery Rev.*, 15, 73-107; Aldrian-Herrada *et al.*, 1998, *Nucleic Acids Res.*, 26, 4910-4916; and Tyler *et al.*, 1999, *PNAS USA.*, 96, 7053-7058.

10 The invention also features the use of the composition comprising surface-modified liposomes containing poly (ethylene glycol) lipids (PEG-modified, or long-circulating liposomes or stealth liposomes). These formulations offer a method for increasing the accumulation of drugs in target tissues. This class of drug carriers resists opsonization and elimination by the mononuclear phagocytic system (MPS or RES),
15 thereby enabling longer blood circulation times and enhanced tissue exposure for the encapsulated drug (Lasic *et al.* *Chem. Rev.* 1995, 95, 2601-2627; Ishiwata *et al.*, *Chem. Pharm. Bull.* 1995, 43, 1005-1011). Such liposomes have been shown to accumulate selectively in tumors, presumably by extravasation and capture in the neovascularized target tissues (Lasic *et al.*, *Science* 1995, 267, 1275-1276; Oku *et al.*, 1995, *Biochim.*
20 *Biophys. Acta*, 1238, 86-90). The long-circulating liposomes enhance the pharmacokinetics and pharmacodynamics of DNA and RNA, particularly compared to conventional cationic liposomes which are known to accumulate in tissues of the MPS (Liu *et al.*, *J. Biol. Chem.* 1995, 270, 24864-24870; Choi *et al.*, International PCT Publication No. WO 96/10391; Ansell *et al.*, International PCT Publication No. WO
25 96/10390; Holland *et al.*, International PCT Publication No. WO 96/10392). Long-circulating liposomes are also likely to protect drugs from nuclease degradation to a greater extent compared to cationic liposomes, based on their ability to avoid accumulation in metabolically aggressive MPS tissues such as the liver and spleen.

The present invention also includes compositions prepared for storage or
30 administration that include a pharmaceutically effective amount of the desired compounds in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or

diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co. (A.R. Gennaro edit. 1985), hereby incorporated by reference herein. For example, preservatives, stabilizers, dyes and flavoring agents can be provided. These include
5 sodium benzoate, sorbic acid and esters of *p*-hydroxybenzoic acid. In addition, antioxidants and suspending agents can be used.

A pharmaceutically effective dose is that dose required to prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state. The pharmaceutically effective dose depends on the type of disease,
10 the composition used, the route of administration, the type of mammal being treated, the physical characteristics of the specific mammal under consideration, concurrent medication, and other factors that those skilled in the medical arts will recognize. Generally, an amount between 0.1 mg/kg and 100 mg/kg body weight/day of active ingredients is administered dependent upon potency of the negatively charged polymer.

15 The nucleic acid molecules of the invention and formulations thereof can be administered orally, topically, parenterally, by inhalation or spray, or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and/or vehicles. The term parenteral as used herein includes percutaneous, subcutaneous, intravascular (e.g., intravenous), intramuscular, or
20 intrathecal injection or infusion techniques and the like. In addition, there is provided a pharmaceutical formulation comprising a nucleic acid molecule of the invention and a pharmaceutically acceptable carrier. One or more nucleic acid molecules of the invention can be present in association with one or more non-toxic pharmaceutically acceptable carriers and/or diluents and/or adjuvants, and if desired other active
25 ingredients. The pharmaceutical compositions containing nucleic acid molecules of the invention can be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs.

Compositions intended for oral use can be prepared according to any method
30 known to the art for the manufacture of pharmaceutical compositions and such compositions can contain one or more such sweetening agents, flavoring agents, coloring

agents or preservative agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients that are suitable for the manufacture of tablets. These excipients can be, for example, inert diluents; such as calcium carbonate, sodium
5 carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia; and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets can be uncoated or they can be coated by known techniques. In some cases such coatings can be prepared by known techniques to delay
10 disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate can be employed.

Formulations for oral use can also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate,
15 calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

Aqueous suspensions contain the active materials in a mixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydropropyl-
20 methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents can be a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products
25 of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions can also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring
30 agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions can be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions can contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents and flavoring agents can be added to provide palatable oral preparations. These compositions can be preserved by the addition of an anti-oxidant such as ascorbic acid

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents or suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, can also be present.

Pharmaceutical compositions of the invention can also be in the form of oil-in-water emulsions. The oily phase can be a vegetable oil or a mineral oil or mixtures of these. Suitable emulsifying agents can be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions can also contain sweetening and flavoring agents.

Syrups and elixirs can be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol, glucose or sucrose. Such formulations can also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions can be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents that have been mentioned above. The sterile injectable preparation can also be a sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any

bland fixed oil can be employed including synthetic mono-or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

5 The nucleic acid molecules of the invention can also be administered in the form of suppositories, *e.g.*, for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient that is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include cocoa butter and polyethylene glycols.

10 Nucleic acid molecules of the invention can be administered parenterally in a sterile medium. The drug, depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as local anesthetics, preservatives and buffering agents can be dissolved in the vehicle.

Dosage levels of the order of from about 0.1 mg to about 140 mg per kilogram of body weight per day are useful in the treatment of the above-indicated conditions (about 15 0.5 mg to about 7 g per subject per day). The amount of active ingredient that can be combined with the carrier materials to produce a single dosage form varies depending upon the host treated and the particular mode of administration. Dosage unit forms generally contain between from about 1 mg to about 500 mg of an active ingredient.

20 It is understood that the specific dose level for any particular subject depends upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, and rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

25 For administration to non-human animals, the composition can also be added to the animal feed or drinking water. It can be convenient to formulate the animal feed and drinking water compositions so that the animal takes in a therapeutically appropriate quantity of the composition along with its diet. It can also be convenient to present the composition as a premix for addition to the feed or drinking water.

The nucleic acid molecules of the present invention can also be administered to a subject in combination with other therapeutic compounds to increase the overall therapeutic effect. The use of multiple compounds to treat an indication can increase the beneficial effects while reducing the presence of side effects.

5 Alternatively, certain siNA molecules of the instant invention can be expressed within cells from eukaryotic promoters (e.g., Izant and Weintraub, 1985, *Science*, 229, 345; McGarry and Lindquist, 1986, *Proc. Natl. Acad. Sci.*, USA 83, 399; Scanlon *et al.*, 1991, *Proc. Natl. Acad. Sci. USA*, 88, 10591-5; Kashani-Sabet *et al.*, 1992, *Antisense Res. Dev.*, 2, 3-15; Dropulic *et al.*, 1992, *J. Virol.*, 66, 1432-41; Weerasinghe *et al.*,
10 1991, *J. Virol.*, 65, 5531-4; Ojwang *et al.*, 1992, *Proc. Natl. Acad. Sci. USA*, 89, 10802-6; Chen *et al.*, 1992, *Nucleic Acids Res.*, 20, 4581-9; Sarver *et al.*, 1990 *Science*, 247, 1222-1225; Thompson *et al.*, 1995, *Nucleic Acids Res.*, 23, 2259; Good *et al.*, 1997, *Gene Therapy*, 4, 45. Those skilled in the art realize that any nucleic acid can be expressed in eukaryotic cells from the appropriate DNA/RNA vector. The activity of
15 such nucleic acids can be augmented by their release from the primary transcript by a enzymatic nucleic acid (Draper *et al.*, PCT WO 93/23569, and Sullivan *et al.*, PCT WO 94/02595; Ohkawa *et al.*, 1992, *Nucleic Acids Symp. Ser.*, 27, 15-6; Taira *et al.*, 1991, *Nucleic Acids Res.*, 19, 5125-30; Ventura *et al.*, 1993, *Nucleic Acids Res.*, 21, 3249-55; Chowrira *et al.*, 1994, *J. Biol. Chem.*, 269, 25856.

20 In another aspect of the invention, RNA molecules of the present invention can be expressed from transcription units (see for example Couture *et al.*, 1996, *TIG.*, 12, 510) inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. In another embodiment,
25 pol III based constructs are used to express nucleic acid molecules of the invention (see for example Thompson, U.S. Pats. Nos. 5,902,880 and 6,146,886). The recombinant vectors capable of expressing the siNA molecules can be delivered as described above, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of nucleic acid molecules. Such vectors can be repeatedly
30 administered as necessary. Once expressed, the siNA molecule interacts with the target mRNA and generates an RNAi response. Delivery of siNA molecule expressing vectors

can be systemic, such as by intravenous or intra-muscular administration, by administration to target cells ex-planted from a subject followed by reintroduction into the subject, or by any other means that would allow for introduction into the desired target cell (for a review see Couture *et al.*, 1996, *TIG.*, 12, 510).

5 In one aspect the invention features an expression vector comprising a nucleic acid sequence encoding at least one siNA molecule of the instant invention. The expression vector can encode one or both strands of a siNA duplex, or a single self-complementary strand that self hybridizes into a siNA duplex. The nucleic acid sequences encoding the siNA molecules of the instant invention can be operably linked in a manner that allows
10 expression of the siNA molecule (see for example Paul *et al.*, 2002, *Nature Biotechnology*, 19, 505; Miyagishi and Taira, 2002, *Nature Biotechnology*, 19, 497; Lee *et al.*, 2002, *Nature Biotechnology*, 19, 500; and Novina *et al.*, 2002, *Nature Medicine*, advance online publication doi:10.1038/nm725).

In another aspect, the invention features an expression vector comprising: a) a
15 transcription initiation region (*e.g.*, eukaryotic pol I, II or III initiation region); b) a transcription termination region (*e.g.*, eukaryotic pol I, II or III termination region); and c) a nucleic acid sequence encoding at least one of the siNA molecules of the instant invention, wherein said sequence is operably linked to said initiation region and said termination region in a manner that allows expression and/or delivery of the siNA
20 molecule. The vector can optionally include an open reading frame (ORF) for a protein operably linked on the 5' side or the 3'-side of the sequence encoding the siNA of the invention; and/or an intron (intervening sequences).

Transcription of the siNA molecule sequences can be driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase
25 III (pol III). Transcripts from pol II or pol III promoters are expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type depends on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990,
30 *Proc. Natl. Acad. Sci. U S A*, 87, 6743-7; Gao and Huang 1993, *Nucleic Acids Res.*, 21, 2867-72; Lieber *et al.*, 1993, *Methods Enzymol.*, 217, 47-66; Zhou *et al.*, 1990, *Mol.*

Cell. Biol., 10, 4529-37). Several investigators have demonstrated that nucleic acid molecules expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet *et al.*, 1992, *Antisense Res. Dev.*, 2, 3-15; Ojwang *et al.*, 1992, *Proc. Natl. Acad. Sci. U S A*, 89, 10802-6; Chen *et al.*, 1992, *Nucleic Acids Res.*, 20, 4581-9; Yu *et al.*, 1993, *Proc. Natl. Acad. Sci. U S A*, 90, 6340-4; L'Huillier *et al.*, 1992, *EMBO J.*, 11, 4411-8; Lisiewicz *et al.*, 1993, *Proc. Natl. Acad. Sci. U. S. A.*, 90, 8000-4; Thompson *et al.*, 1995, *Nucleic Acids Res.*, 23, 2259; Sullenger & Cech, 1993, *Science*, 262, 1566). More specifically, transcription units such as the ones derived from genes encoding U6 small nuclear (snRNA), transfer RNA (tRNA) and adenovirus VA RNA are useful in generating high concentrations of desired RNA molecules such as siNA in cells (Thompson *et al.*, *supra*; Couture and Stinchcomb, 1996, *supra*; Noonberg *et al.*, 1994, *Nucleic Acid Res.*, 22, 2830; Noonberg *et al.*, U.S. Pat. No. 5,624,803; Good *et al.*, 1997, *Gene Ther.*, 4, 45; Beigelman *et al.*, International PCT Publication No. WO 96/18736. The above siNA transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors) (for a review see Couture and Stinchcomb, 1996, *supra*).

In another aspect the invention features an expression vector comprising a nucleic acid sequence encoding at least one of the siNA molecules of the invention in a manner that allows expression of that siNA molecule. The expression vector comprises in one embodiment; a) a transcription initiation region; b) a transcription termination region; and c) a nucleic acid sequence encoding at least one strand of the siNA molecule, wherein the sequence is operably linked to the initiation region and the termination region in a manner that allows expression and/or delivery of the siNA molecule.

In another embodiment the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an open reading frame; and d) a nucleic acid sequence encoding at least one strand of a siNA molecule, wherein the sequence is operably linked to the 3'-end of the open reading frame and wherein the sequence is operably linked to the initiation region, the open reading frame and the termination region in a manner that allows expression and/or delivery of the siNA

molecule. In yet another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; and d) a nucleic acid sequence encoding at least one siNA molecule, wherein the sequence is operably linked to the initiation region, the intron and the termination region in a manner
5 which allows expression and/or delivery of the nucleic acid molecule.

In another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; d) an open reading frame; and e) a nucleic acid sequence encoding at least one strand of a siNA molecule, wherein the sequence is operably linked to the 3'-end of the open reading frame and
10 wherein the sequence is operably linked to the initiation region, the intron, the open reading frame and the termination region in a manner which allows expression and/or delivery of the siNA molecule.

Parkinson's disease genetics, biology and biochemistry

The following entire discussion is adapted from Dawson and Dawson, 2003, *J. Clin. Invest.*, 111, 145-151 and references provided therein, which provides an excellent
15 overview of the genetic and pathogenic basis of Parkinson's disease including the role of various genes (PARK1, PARK2, PARK5, and PARK7) in disease progression and pathology. Parkinson's disease (PD) is the second most common progressive neurodegenerative disorder next to Alzheimer's disease, with a prevalence of
20 approximately 1% at the age of 65, increasing to 4-5% by the age of 85. PD patients suffer from various motor dysfunctions, including bradykinesia, tremor, cogwheel rigidity, and postural instability. Cognitive dysfunction (dementia) is also apparent in PD as well. PD manifests from the selective loss of dopaminergic neurons in the substantia nigra pars compacta, which leads to a profound reduction in striatal dopamine (DA).
25 Aggregates of Lewy bodies and dystrophic neurites (Lewy neurites) tend to accompany the loss of dopaminergic neurons. Similar to beta-amyloid plaques seen in Alzheimer's disease, Lewy bodies are a pathologic hallmark of PD which are round eosinophilic inclusions composed of a halo of radiating fibrils and a less defined core. Both Lewy bodies and Lewy neurites are comprised of cytoplasmic accumulations of aggregated
30 proteins. PD represents a heterogeneous disorder with common clinical manifestations and, for the most part, common neuropathologic findings. The majority of cases of PD

appear to be sporadic in nature; however, there may be genetic risk factors that increase the likelihood of developing PD, much in the same way that the apoE4 allele increases the risk of developing Alzheimer disease (AD). Familial PD with specific genetic defects may account for fewer than 10% of all cases of PD; however, the identification of these rare genes and their functions has provided tremendous insight into the pathogenesis of PD and opened up new areas of investigation.

Three genes have been clearly linked to PD, and a number of other genes or genetic linkages have been identified that may cause PD. The first PD gene to be identified, PARK1, was the gene encoding the presynaptic protein alpha-synuclein (SNCA). The second PD gene, PARK2, is caused by mutations in the gene for parkin, and it leads to autosomal recessive juvenile parkinsonism (AR-JP). The third PD gene, PARK7, results from mutations in DJ-1. Mutations in alpha-synuclein, parkin, and DJ-1 are thought to cause PD. A mutation in the gene (PARK5) encoding ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) in two family members of a small German kindred with autosomal dominant PD has also been described (see Roses, 1998, *Ann. NY Acad. Sci.*, 855, 738-743).

A locus located on chromosome 2p13 (PARK3) has been described in a subset of families with autosomal dominant inheritance and typical Lewy body pathology. The penetrance of the mutation on chromosome 2p13 was estimated to be 40% based on the occurrence of the affected haplotype in clinically asymptomatic members of the linked families. PARK3-linked families also show signs of dementia, and neuropathology revealed, in addition to neuronal loss in the substantia nigra and typical brainstem Lewy bodies, the presence of neurofibrillary tangles and Alzheimer plaques. PARK4 is linked to the short arm of chromosome 4 (4p15). The PARK4 locus appears to segregate with both PD and postural tremor with an autosomal dominant inheritance pattern. Furthermore, affected family members with PD have several atypical features, including early weight loss, dysautonomia, and dementia. An autosomal recessive locus on chromosome 1, PARK6, has recently been described in a large Sicilian family and is linked to chromosome 1 (1p35-p36). Linkage analysis in a consanguineous family from the southwest Netherlands revealed another locus (PARK7) on chromosome 1p36, which is genetically and clinically distinct from PARK6. PARK8 is linked to chromosome

12p11.2-q13.1 and is inherited in an autosomal dominant fashion with partial penetrance. Neuropathologic examination of four patients revealed nigral degeneration without Lewy bodies. Kufor-Rakeb syndrome, an autosomal recessive nigro-striatal-pallidal-pyramidal neurodegenerative disorder, has been mapped to a 9-cM region of chromosome 1p36 and designated PARK9. A recent Icelandic study suggests that genetic variability is a major contributor to PD in this population, and the locus was recently localized to a region on chromosome 1p32 and designated PARK10. It is likely that there are other gene loci, as not all familial PD has been linked to the current loci. The identification of the genes for PARK1 (alpha-synuclein), PARK2 (parkin), and PARK7 (DJ-1) has led to new insights and direction in PD research and pathogenesis.

Besides identifying genetic mutations that cause PD, recent genomic screens have also identified genetic factors that may be important in its development. In particular, linkage and mutation analysis indicates that the parkin gene, in addition to being a direct cause of PD, is influential in the development of early-onset PD. Multiple genetic factors appear to be important in the development of idiopathic late-onset PD. Four single-nucleotide polymorphisms in the tau gene are significantly associated with an increased risk of developing PD. Thus, the association of PD with the haplotype of tau and the evidence for linkage to that region of chromosome 17q suggest that tau, or a gene in linkage disequilibrium with tau, is a genetic risk factor for PD. Three large case-series studies also established a significant association between polymorphism of the tau gene and PD. Frontotemporal dementia with parkinsonism (FTDP) is caused, in part, by mutations in tau. Chromosome 9q also seems to be a region that incurs a genetic risk factor for PD. Other suggestive linkages have been identified on chromosomes 1, 3q, 5q, 8p, 10, and 16. Genes influencing the age of onset of PD may be linked to chromosome 1p and chromosomes 6 and 10. Interestingly, the linkage on chromosome 10 also influences the age of onset of AD.

Mutations in the alpha-synuclein gene are a cause of some forms of PD. The first mutation identified was an A53T mutation resulting from a G to A transition at position 209. This mutation was originally found in the Contursi kindred and was also identified in several Greek kindreds. Recent haplotype analyses suggest that the Contursi kindred and the Greek kindreds share a common ancestor. Another mutation (A30P) resulting

from a G to C transition at position 88 was identified in a small German kindred. Furthermore, genetic variability in the alpha-synuclein gene is a risk factor for the development of PD. Affected individuals with alpha-synuclein mutations have typical idiopathic PD, including levodopa responsiveness and Lewy bodies, although the age of onset is somewhat lower and progression appears to be more rapid. Alpha-synuclein is a 140-amino acid protein that contains repetitive imperfect repeats of KTKEGV in the amino-terminal half, a hydrophobic region, and an acidic carboxy-terminal region. In humans, there are at least three different synuclein family members, designated alpha, beta, and gamma-synuclein, and they are expressed from three different genes. Synucleins are abundant brain proteins whose physiologic functions are poorly understood. Alpha-synuclein has been shown to bind to a number of proteins as well as lipid membranes. It has been suggested that alpha-synuclein may play some role in the modulation of synaptic vesicle turnover and synaptic plasticity. Alpha-synuclein knockout mice are viable and fertile and exhibit normal brain structure and a normal complement of dopaminergic cell bodies, fibers, and synapses. Thus, a loss of function in alpha-synuclein is unlikely to cause PD, and mutations in alpha-synuclein that cause PD are likely to be gain-of-function mutations. Alpha-synuclein knockout mice have increased DA release following paired stimuli and an attenuation of DA-dependent locomotor responses to amphetamine, which suggest that alpha-synuclein may be an essential presynaptic, activity-dependent negative regulator of dopaminergic neurotransmission. Alpha-synuclein appears to be the primary component of the Lewy body. It has the ability to polymerize into approximately 10-nm fibrils in vitro, and bundles of these fibrils are the major component of Lewy bodies and Lewy neurites.

Overexpression of human wild-type alpha-synuclein in mice using the PDGF promoter yielded mice with selective decrements in DA nerve terminals in the striatum, with a concomitant reduction in tyrosine hydroxylase catalytic activity. A variety of transgenic mice overexpressing wild-type or mutant forms of alpha-synuclein have been described with varying degrees of pathology and alpha-synuclein abnormalities. None of the mammalian transgenic models fully recapitulate PD, but they have proved useful for studying synucleinopathy-induced neurodegeneration. Alpha-synuclein dependent neurodegeneration is associated with abnormal accumulation of detergent-insoluble alpha-synuclein, and abnormal proteolytic processing of alpha-synuclein and the A53T

alpha-synuclein mutant appears to cause significantly greater in vivo toxicity as compared with the other alpha-synuclein variants. Beta-Synuclein, the nonamyloidogenic homologue of alpha-synuclein, is an inhibitor of aggregation of alpha-synuclein and rescues the motor deficits, neurodegenerative alterations, and neuronal alpha-synuclein accumulations seen in human PDGF-promoter alpha-synuclein transgenic mice. Thus, beta-synuclein might be a neutral negative regulator of alpha-synuclein aggregation, and the antiamyloidogenic property of beta-synuclein may provide a novel strategy for the treatment of neurodegenerative disorders. In addition to the mammalian models, *Drosophila* models have been developed. When normal and mutant forms of alpha-synuclein are overexpressed in *Drosophila*, the flies develop an adult-onset (midlife) progressive loss of DA neurons and filamentous interneuronal inclusions that contain alpha-synuclein. Overexpression of heat-shock protein HSP70 rescues the motoric and neuropathologic features of transgenic flies expressing normal and mutant forms of alpha-synuclein. Chaperones may play a role in PD, as Lewy bodies in human postmortem tissue immunostain for chaperones. The transgenic mouse and *Drosophila* models are persuasive in implicating alpha-synuclein in the pathogenesis of PD.

Linkage analysis of 13 families with AR-JP mapped the localization of the AR-JP gene to chromosome 6q25.2-27. The identification of the AR-JP gene was facilitated by the discovery of a microdeletion in a family with AR-JP. The gene causing AR-JP was designated parkin and encodes a protein of 465 amino acids, with moderate similarity to ubiquitin at its amino-terminus and a RING-finger motif at the carboxy-terminus. In the patient with a microdeletion, exons 3-7 were deleted, and four other AR-JP patients from three unrelated families also had a deletion affecting exon 4. Since the initial discovery of the parkin gene, many groups have identified mutations in parkin, including exonic deletions, insertions, and several missense mutations. So far, most of the point mutations reside in the RING-IBR-RING domains of parkin, suggesting that this region is key to parkin function. Mutations in parkin appear to be a major cause of autosomal recessive PD. Indeed, parkin mutations are now considered to be one of the major causes of familial PD.

The modular architecture of parkin led to insight into its function, as several other proteins had similar modular structures. In particular, a few proteins with RING-finger

motifs were shown to be involved in E2-dependent ubiquitination. Proteins are targeted for degradation in the 20S proteasome by covalent attachment of ubiquitin. The 26S proteasome recognizes multiubiquitin chains that are formed by linkage through the lysine residue at position 48 in the ubiquitin protein. Polyubiquitination occurs by the cooperation of several sequentially acting enzymes. The ubiquitin-activating enzyme E1 activates the ubiquitin in an ATP-dependent manner; then ubiquitin is transferred to a ubiquitin-conjugating enzyme, E2. A final step requires an E3 ubiquitin-protein ligase, which facilitates the transfer of ubiquitin to the target protein. Substrate specificity of the ubiquitin system is largely conferred by the E3 ubiquitin-protein ligase. Parkin was shown to be an E2-dependent E3 ubiquitin-protein ligase. It appears to use both UbcH7 and UbcH8 as its E2s, and it also utilizes the ER-associated E2s UBC6 and UBC7. Familial-associated mutations in parkin impair the binding to either UbcH7 or UbcH8 and are defective in E3 ubiquitin-protein ligase activity, which suggests that disruption of the E3 ubiquitin-protein ligase activity of parkin is probably the cause of autosomal recessive PD. Since the loss of the E3 ligase activity of parkin may cause autosomal recessive PD, it is of great importance to identify the protein substrates of parkin. It is conceivable that dysfunction of the proteasomal processing of one or more of these proteins leads to dopaminergic dysfunction. Several potential substrates for parkin have recently been identified. The first substrate identified was the synaptic vesicle-associated protein CDCrel-1. CDCrel-1 belongs to a family of septin GTPases, and it has been suggested that it regulates synaptic vesicle release in the nervous system. Whether CDCrel-1 is involved in the release of DA is not yet known, but it is possible that mutations in parkin affect CDCrel-1 modulation of DA release, which ultimately contributes to the parkinsonian state.

Synphilin-1 is also a substrate for parkin-targeted ubiquitination. The function of synphilin-1 is unknown, but it was identified and cloned as an alpha-synuclein-interacting protein. It is also a synaptic vesicle-enriched protein, and it is present in Lewy bodies. Coexpression of synphilin-1 with alpha-synuclein in cultured cells results in the formation of Lewy body-like aggregates containing both proteins; and in the presence of parkin, a significant percentage of these aggregates become ubiquitinated. The observation that patients with mutations in parkin do not have Lewy bodies has led to the speculation that pathogenic mechanisms caused by mutations in parkin are different from

those that occur in sporadic PD and in PD due to mutations in alpha-synuclein. On the other hand, the interactions of synphilin-1 with both parkin and alpha-synuclein suggest a common link between the different causes of PD and connect the pathogenesis of PD caused by mutations in parkin with that of PD caused by alterations in alpha-synuclein.

5 Parkin may be intimately involved in the ubiquitination of Lewy body-associated proteins, such as synphilin-1. Furthermore, the sequestration of parkin in inclusions may contribute to its loss of function. In the absence of parkin, Lewy body-associated proteins would not be ubiquitinated and the formation of Lewy bodies would be impaired. Consistent with this notion is the observation that familial-associated mutations
10 of parkin fail to ubiquitinate synphilin-1. Recently, a patient with an R275W mutation in one allele of parkin and a 40-bp exon 3 deletion in the other allele revealed the presence of Lewy body pathology in regions typically affected in PD. Interestingly, the R275W parkin mutation reduces the catalytic activity of parkin, but it still has substantial enzyme activity. Thus, this mutation appears to be the exception that proves the rule and indicates
15 that parkin is required for the formation of Lewy pathology. Lewy pathology may contribute, in part, to neuronal cell death by the sequestration of the function of parkin, which serves to degrade specific proteins. Using immunological methods in the normal human brain, Shimura et al. identified an O-glycosylated isoform of alpha-synuclein (alpha-Sp22) that contains complex monosaccharide chains. Familial-associated parkin
20 mutants failed to bind alpha-Sp22, and, in an in vitro ubiquitination assay, alpha-Spp22 was ubiquitinated by normal, but not by mutant, parkin.

A more general role for parkin in the ubiquitin proteasomal-degradation pathway is suggested by the recent observations that parkin is upregulated by unfolded-protein stress. Parkin has been found to suppress unfolded protein-stress induced toxicity. Parkin
25 may function in the unfolded-protein response, as it is localized to the microsomal fraction as well as to the cytosol and Golgi fractions. The unfolded-protein response regulates multiple ER and secretory pathway genes, and it is conceivable that mutations or deletions of the parkin gene could result in the accumulation of misfolded substrate proteins in the ER, leading to DA cell death in AR-JP. Recently, an unfolded putative G
30 protein-coupled transmembrane receptor, the parkin-associated endothelial-like receptor (Pael-R), was found to be a parkin substrate. When overexpressed, Pael-R tends to become unfolded and insoluble and causes unfolded protein induced cell death. Parkin

ubiquitinates Pael-R, and coexpression of parkin results in protection against Pael-R induced cell toxicity. Pael-R accumulates in the brains of AR-JP patients and thus may be an important parkin substrate. In the brain, Pael-R is expressed predominantly in oligodendrocytes, but it is also expressed at exceptionally high levels in neurons
5 containing tyrosine hydroxylase. Thus, Pael-R is an attractive parkin substrate whose accumulation may account for the loss of DA neurons in AR-JP.

In genetically isolated communities in the Netherlands, Bonifati and colleagues, using an RT-PCR strategy, identified a deletion in exons 1^{AB} to 5 of the DJ-1 gene that showed complete cosegregation with PD and the disease allele in a Dutch family (see
10 Bonifati, *et al.*, 2002, *Science*, doi:10.1126/science.1077209). In addition, a T to C transition at position 497 from the open reading frame start in the cDNA of DJ-1 resulting in the substitution of a highly conserved leucine at position 166 of the DJ-1 protein by a proline was identified that shows complete cosegregation with the disease allele in an Italian family. In the Dutch family the DJ-1 protein is absent, and in the
15 Italian family DJ-1 appears to be functionally inactive. Thus, mutations in the DJ-1 gene cause PD, likely through a loss of function. It is difficult at this juncture to fully appreciate how mutations in the DJ-1 gene cause PD, as its function is largely unknown. However, DJ-1 was identified as a hydroperoxide-responsive protein that becomes more acidic following oxidative stress, suggesting that it may function as an antioxidant
20 protein. Furthermore, DJ-1 is sumoylated through binding to the SUMO-1 ligase PIAS, suggesting that it might be involved in the regulation of transcription.

Examples:

The following are non-limiting examples showing the selection, isolation, synthesis and activity of nucleic acids of the instant invention.

25 Example 1: Tandem synthesis of siNA constructs

Exemplary siNA molecules of the invention are synthesized in tandem using a cleavable linker, for example, a succinyl-based linker. Tandem synthesis as described herein is followed by a one-step purification process that provides RNAi molecules in high yield. This approach is highly amenable to siNA synthesis in support of high

throughput RNAi screening, and can be readily adapted to multi-column or multi-well synthesis platforms.

After completing a tandem synthesis of a siNA oligo and its complement in which the 5'-terminal dimethoxytrityl (5'-O-DMT) group remains intact (trityl on synthesis), the oligonucleotides are deprotected as described above. Following deprotection, the siNA sequence strands are allowed to spontaneously hybridize. This hybridization yields a duplex in which one strand has retained the 5'-O-DMT group while the complementary strand comprises a terminal 5'-hydroxyl. The newly formed duplex behaves as a single molecule during routine solid-phase extraction purification (Trityl-On purification) even though only one molecule has a dimethoxytrityl group. Because the strands form a stable duplex, this dimethoxytrityl group (or an equivalent group, such as other trityl groups or other hydrophobic moieties) is all that is required to purify the pair of oligos, for example, by using a C18 cartridge.

Standard phosphoramidite synthesis chemistry is used up to the point of introducing a tandem linker, such as an inverted deoxy abasic succinate or glyceryl succinate linker (see Figure 1) or an equivalent cleavable linker. A non-limiting example of linker coupling conditions that can be used includes a hindered base such as diisopropylethylamine (DIPA) and/or DMAP in the presence of an activator reagent such as Bromotripyrrolidinophosphoniumhexafluorophosphate (PyBrOP). After the linker is coupled, standard synthesis chemistry is utilized to complete synthesis of the second sequence leaving the terminal the 5'-O-DMT intact. Following synthesis, the resulting oligonucleotide is deprotected according to the procedures described herein and quenched with a suitable buffer, for example with 50mM NaOAc or 1.5M $\text{NH}_4\text{H}_2\text{CO}_3$.

Purification of the siNA duplex can be readily accomplished using solid phase extraction, for example using a Waters C18 SepPak 1g cartridge conditioned with 1 column volume (CV) of acetonitrile, 2 CV H_2O , and 2 CV 50mM NaOAc. The sample is loaded and then washed with 1 CV H_2O or 50mM NaOAc. Failure sequences are eluted with 1 CV 14% ACN (Aqueous with 50mM NaOAc and 50mM NaCl). The column is then washed, for example with 1 CV H_2O followed by on-column detritylation, for example by passing 1 CV of 1% aqueous trifluoroacetic acid (TFA)

over the column, then adding a second CV of 1% aqueous TFA to the column and allowing to stand for approximately 10 minutes. The remaining TFA solution is removed and the column washed with H₂O followed by 1 CV 1M NaCl and additional H₂O. The siNA duplex product is then eluted, for example, using 1 CV 20% aqueous
5 CAN.

Figure 2 provides an example of MALDI-TOF mass spectrometry analysis of a purified siNA construct in which each peak corresponds to the calculated mass of an individual siNA strand of the siNA duplex. The same purified siNA provides three peaks when analyzed by capillary gel electrophoresis (CGE), one peak presumably
10 corresponding to the duplex siNA, and two peaks presumably corresponding to the separate siNA sequence strands. Ion exchange HPLC analysis of the same siNA contract only shows a single peak. Testing of the purified siNA construct using a luciferase reporter assay described below demonstrated the same RNAi activity compared to siNA constructs generated from separately synthesized oligonucleotide sequence strands.

15 Example 2: Identification of potential siNA target sites in any RNA sequence

The sequence of an RNA target of interest, such as a viral or human mRNA transcript, is screened for target sites, for example by using a computer folding algorithm. In a non-limiting example, the sequence of a gene or RNA gene transcript derived from a database, such as Genbank, is used to generate siNA targets having
20 complementarity to the target. Such sequences can be obtained from a database, or can be determined experimentally as known in the art. Target sites that are known, for example, those target sites determined to be effective target sites based on studies with other nucleic acid molecules, for example ribozymes or antisense, or those targets known to be associated with a disease or condition such as those sites containing mutations or
25 deletions, can be used to design siNA molecules targeting those sites. Various parameters can be used to determine which sites are the most suitable target sites within the target RNA sequence. These parameters include but are not limited to secondary or tertiary RNA structure, the nucleotide base composition of the target sequence, the degree of homology between various regions of the target sequence, or the relative
30 position of the target sequence within the RNA transcript. Based on these determinations, any number of target sites within the RNA transcript can be chosen to

- screen siNA molecules for efficacy, for example by using *in vitro* RNA cleavage assays, cell culture, or animal models. In a non-limiting example, anywhere from 1 to 1000 target sites are chosen within the transcript based on the size of the siNA construct to be used. High throughput screening assays can be developed for screening siNA molecules
- 5 using methods known in the art, such as with multi-well or multi-plate assays to determine efficient reduction in target gene expression.

Example 3: Selection of siNA molecule target sites in a RNA

The following non-limiting steps can be used to carry out the selection of siNAs targeting a given gene sequence or transcript.

- 10 1. The target sequence is parsed *in silico* into a list of all fragments or subsequences of a particular length, for example 23 nucleotide fragments, contained within the target sequence. This step is typically carried out using a custom Perl script, but commercial sequence analysis programs such as Oligo, MacVector, or the GCG Wisconsin Package can be employed as well.
- 15 2. In some instances the siNAs correspond to more than one target sequence; such would be the case for example in targeting different transcripts of the same gene, targeting different transcripts of more than one gene, or for targeting both the human gene and an animal homolog. In this case, a subsequence list of a particular length is generated for each of the targets, and then the lists are compared to find matching
- 20 sequences in each list. The subsequences are then ranked according to the number of target sequences that contain the given subsequence; the goal is to find subsequences that are present in most or all of the target sequences. Alternately, the ranking can identify subsequences that are unique to a target sequence, such as a mutant target sequence. Such an approach would enable the use of siNA to target specifically the
- 25 mutant sequence and not effect the expression of the normal sequence.
3. In some instances the siNA subsequences are absent in one or more sequences while present in the desired target sequence; such would be the case if the siNA targets a gene with a paralogous family member that is to remain untargeted. As in case 2 above, a subsequence list of a particular length is generated for each of the targets,

and then the lists are compared to find sequences that are present in the target gene but are absent in the untargeted paralog.

4. The ranked siNA subsequences can be further analyzed and ranked according to GC content. A preference can be given to sites containing 30-70% GC, with a further
5 preference to sites containing 40-60% GC.
5. The ranked siNA subsequences can be further analyzed and ranked according to self-folding and internal hairpins. Weaker internal folds are preferred; strong hairpin structures are to be avoided.
6. The ranked siNA subsequences can be further analyzed and ranked according to
10 whether they have runs of GGG or CCC in the sequence. GGG (or even more Gs) in either strand can make oligonucleotide synthesis problematic and can potentially interfere with RNAi activity, so it is avoided whenever better sequences are available. CCC is searched in the target strand because that will place GGG in the antisense strand.
- 15 7. The ranked siNA subsequences can be further analyzed and ranked according to whether they have the dinucleotide UU (uridine dinucleotide) on the 3'-end of the sequence, and/or AA on the 5'-end of the sequence (to yield 3' UU on the antisense sequence). These sequences allow one to design siNA molecules with terminal TT thymidine dinucleotides.
- 20 8. Four or five target sites are chosen from the ranked list of subsequences as described above. For example, in subsequences having 23 nucleotides, the right 21 nucleotides of each chosen 23-mer subsequence are then designed and synthesized for the upper (sense) strand of the siNA duplex, while the reverse complement of the left 21 nucleotides of each chosen 23-mer subsequence are then designed and synthesized
25 for the lower (antisense) strand of the siNA duplex (see Tables II and III). If terminal TT residues are desired for the sequence (as described in paragraph 7), then the two 3' terminal nucleotides of both the sense and antisense strands are replaced by TT prior to synthesizing the oligos.

9. The siNA molecules are screened in an *in vitro*, cell culture or animal model system to identify the most active siNA molecule or the most preferred target site within the target RNA sequence.
10. Other design considerations can be used when selecting target nucleic acid sequences, see for example Reynolds *et al.*, 2004, *Nature Biotechnology Advanced Online Publication*, 1 February 2004, doi:10.1038/nbt936 and Ui-Tei *et al.*, 2004, *Nucleic Acids Research*, 32, doi:10.1093/nar/gkh247.

In an alternate approach, a pool of siNA constructs specific to a PARK (e.g., SNCA) target sequence is used to screen for target sites in cells expressing SNCA (or other PARK gene) RNA, such as ecdysone-inducible neuro2a cells (see for example Iwata *et al.*, 2001, *J. Biol. Chem.*, 276, 45320-9) or engineered PC12 cells (see for example Lee *et al.*, 2003, *Neurobiol. Aging*, 24, 687-696). The general strategy used in this approach is shown in Figure 9. A non-limiting example of such is a pool comprising sequences having any of SEQ ID NOS 1-370. Cells expressing SNCA (or other PARK gene RNA) are transfected with the pool of siNA constructs and cells that demonstrate a phenotype associated with SNCA (or other PARK gene) inhibition are sorted. The pool of siNA constructs can be expressed from transcription cassettes inserted into appropriate vectors (see for example Figure 7 and Figure 8). The siNA from cells demonstrating a positive phenotypic change (e.g., decreased proliferation, decreased PARK (e.g., SNCA) mRNA levels or decreased PARK (e.g., SNCA) protein expression), are sequenced to determine the most suitable target site(s) within the target PARK (e.g., SNCA) RNA sequence.

Example 4: PARK (e.g., SNCA) targeted siNA design

siNA target sites were chosen by analyzing sequences of the PARK (e.g., SNCA) RNA target and optionally prioritizing the target sites on the basis of folding (structure of any given sequence analyzed to determine siNA accessibility to the target), by using a library of siNA molecules as described in Example 3, or alternately by using an *in vitro* siNA system as described in Example 6 herein. siNA molecules were designed that could bind each target and are optionally individually analyzed by computer folding to assess whether the siNA molecule can interact with the target sequence. Varying the

length of the siNA molecules can be chosen to optimize activity. Generally, a sufficient number of complementary nucleotide bases are chosen to bind to, or otherwise interact with, the target RNA, but the degree of complementarity can be modulated to accommodate siNA duplexes or varying length or base composition. By using such methodologies, siNA molecules can be designed to target sites within any known RNA sequence, for example those RNA sequences corresponding to the any gene transcript.

Chemically modified siNA constructs are designed to provide nuclease stability for systemic administration in vivo and/or improved pharmacokinetic, localization, and delivery properties while preserving the ability to mediate RNAi activity. Chemical modifications as described herein are introduced synthetically using synthetic methods described herein and those generally known in the art. The synthetic siNA constructs are then assayed for nuclease stability in serum and/or cellular/tissue extracts (e.g. liver extracts). The synthetic siNA constructs are also tested in parallel for RNAi activity using an appropriate assay, such as a luciferase reporter assay as described herein or another suitable assay that can quantify RNAi activity. Synthetic siNA constructs that possess both nuclease stability and RNAi activity can be further modified and re-evaluated in stability and activity assays. The chemical modifications of the stabilized active siNA constructs can then be applied to any siNA sequence targeting any chosen RNA and used, for example, in target screening assays to pick lead siNA compounds for therapeutic development (see for example Figure 11).

Example 5: Chemical Synthesis and Purification of siNA

siNA molecules can be designed to interact with various sites in the RNA message, for example, target sequences within the RNA sequences described herein. The sequence of one strand of the siNA molecule(s) is complementary to the target site sequences described above. The siNA molecules can be chemically synthesized using methods described herein. Inactive siNA molecules that are used as control sequences can be synthesized by scrambling the sequence of the siNA molecules such that it is not complementary to the target sequence. Generally, siNA constructs can be synthesized using solid phase oligonucleotide synthesis methods as described herein (see for example Usman *et al.*, US Patent Nos. 5,804,683; 5,831,071; 5,998,203; 6,117,657; 6,353,098;

6,362,323; 6,437,117; 6,469,158; Scaringe *et al.*, US Patent Nos. 6,111,086; 6,008,400; 6,111,086 all incorporated by reference herein in their entirety).

In a non-limiting example, RNA oligonucleotides are synthesized in a stepwise fashion using the phosphoramidite chemistry as is known in the art. Standard
5 phosphoramidite chemistry involves the use of nucleosides comprising any of 5'-O-dimethoxytrityl, 2'-O-tert-butyldimethylsilyl, 3'-O-2-Cyanoethyl N,N-diisopropylphosphoroamidite groups, and exocyclic amine protecting groups (e.g. N6-benzoyl adenosine, N4 acetyl cytidine, and N2-isobutyryl guanosine). Alternately, 2'-O-Silyl Ethers can be used in conjunction with acid-labile 2'-O-orthoester protecting groups in the synthesis of
10 RNA as described by Scaringe *supra*. Differing 2' chemistries can require different protecting groups, for example 2'-deoxy-2'-amino nucleosides can utilize N-phthaloyl protection as described by Usman *et al.*, US Patent 5,631,360, incorporated by reference herein in its entirety).

During solid phase synthesis, each nucleotide is added sequentially (3'- to 5'-
15 direction) to the solid support-bound oligonucleotide. The first nucleoside at the 3'-end of the chain is covalently attached to a solid support (e.g., controlled pore glass or polystyrene) using various linkers. The nucleotide precursor, a ribonucleoside phosphoramidite, and activator are combined resulting in the coupling of the second nucleoside phosphoramidite onto the 5'-end of the first nucleoside. The support is then
20 washed and any unreacted 5'-hydroxyl groups are capped with a capping reagent such as acetic anhydride to yield inactive 5'-acetyl moieties. The trivalent phosphorus linkage is then oxidized to a more stable phosphate linkage. At the end of the nucleotide addition cycle, the 5'-O-protecting group is cleaved under suitable conditions (e.g., acidic conditions for trityl-based groups and Fluoride for silyl-based groups). The cycle is
25 repeated for each subsequent nucleotide.

Modification of synthesis conditions can be used to optimize coupling efficiency, for example by using differing coupling times, differing reagent/phosphoramidite concentrations, differing contact times, differing solid supports and solid support linker chemistries depending on the particular chemical composition of the siNA to be
30 synthesized. Deprotection and purification of the siNA can be performed as is generally described in Deprotection and purification of the siNA can be performed as is generally

described in Usman *et al.*, US 5,831,071, US 6,353,098, US 6,437,117, and Bellon *et al.*, US 6,054,576, US 6,162,909, US 6,303,773, or Scaringe *supra*, incorporated by reference herein in their entireties. Additionally, deprotection conditions can be modified to provide the best possible yield and purity of siNA constructs. For example, applicant has observed that oligonucleotides comprising 2'-deoxy-2'-fluoro nucleotides can degrade under inappropriate deprotection conditions. Such oligonucleotides are deprotected using aqueous methylamine at about 35°C for 30 minutes. If the 2'-deoxy-2'-fluoro containing oligonucleotide also comprises ribonucleotides, after deprotection with aqueous methylamine at about 35°C for 30 minutes, TEA-HF is added and the reaction maintained at about 65°C for an additional 15 minutes.

Example 6: RNAi *in vitro* assay to assess siNA activity

An *in vitro* assay that recapitulates RNAi in a cell-free system is used to evaluate siNA constructs targeting PARK (e.g., SNCA) RNA targets. The assay comprises the system described by Tuschl *et al.*, 1999, *Genes and Development*, 13, 3191-3197 and Zamore *et al.*, 2000, *Cell*, 101, 25-33 adapted for use with PARK (e.g., SNCA) target RNA. A *Drosophila* extract derived from syncytial blastoderm is used to reconstitute RNAi activity *in vitro*. Target RNA is generated via *in vitro* transcription from an appropriate PARK (e.g., SNCA) expressing plasmid using T7 RNA polymerase or via chemical synthesis as described herein. Sense and antisense siNA strands (for example 20 uM each) are annealed by incubation in buffer (such as 100 mM potassium acetate, 30 mM HEPES-KOH, pH 7.4, 2 mM magnesium acetate) for 1 minute at 90°C followed by 1 hour at 37°C, then diluted in lysis buffer (for example 100 mM potassium acetate, 30 mM HEPES-KOH at pH 7.4, 2mM magnesium acetate). Annealing can be monitored by gel electrophoresis on an agarose gel in TBE buffer and stained with ethidium bromide. The *Drosophila* lysate is prepared using zero to two-hour-old embryos from Oregon R flies collected on yeasted molasses agar that are dechorionated and lysed. The lysate is centrifuged and the supernatant isolated. The assay comprises a reaction mixture containing 50% lysate [vol/vol], RNA (10-50 pM final concentration), and 10% [vol/vol] lysis buffer containing siNA (10 nM final concentration). The reaction mixture also contains 10 mM creatine phosphate, 10 ug/ml creatine phosphokinase, 100 uM GTP, 100 uM UTP, 100 uM CTP, 500 uM ATP, 5 mM DTT, 0.1 U/uL RNasin (Promega), and 100

uM of each amino acid. The final concentration of potassium acetate is adjusted to 100 mM. The reactions are pre-assembled on ice and preincubated at 25° C for 10 minutes before adding RNA, then incubated at 25° C for an additional 60 minutes. Reactions are quenched with 4 volumes of 1.25 x Passive Lysis Buffer (Promega). Target RNA
5 cleavage is assayed by RT-PCR analysis or other methods known in the art and are compared to control reactions in which siNA is omitted from the reaction.

Alternately, internally-labeled target RNA for the assay is prepared by *in vitro* transcription in the presence of [α -³²P] CTP, passed over a G 50 Sephadex column by spin chromatography and used as target RNA without further purification.
10 Optionally, target RNA is 5'-³²P-end labeled using T4 polynucleotide kinase enzyme. Assays are performed as described above and target RNA and the specific RNA cleavage products generated by RNAi are visualized on an autoradiograph of a gel. The percentage of cleavage is determined by PHOSPHOR IMAGER® (autoradiography) quantitation of bands representing intact control RNA or RNA from control reactions
15 without siNA and the cleavage products generated by the assay.

In one embodiment, this assay is used to determine target sites the PARK (e.g., SNCA) RNA target for siNA mediated RNAi cleavage, wherein a plurality of siNA constructs are screened for RNAi mediated cleavage of the PARK (e.g., SNCA) RNA target, for example, by analyzing the assay reaction by electrophoresis of labeled target
20 RNA, or by northern blotting, as well as by other methodology well known in the art.

In a non-limiting example, siNA constructs targeting SNCA RNA transcripts were assayed in an *in vitro* assay that recapitulates RNAi in a cell-free system. Lysate derived from HeLa cells was used to reconstitute RNAi activity *in vitro*. Target RNA was generated via *in vitro* transcription from an appropriate SNCA RNA expressing dsDNA
25 using T7 RNA polymerase. Sense and antisense siNA strands (20 uM each) were annealed by incubation in buffer (100 mM potassium acetate, 30 mM HEPES-KOH, pH 7.4, 2 mM magnesium acetate) for 1 minute at 90°C followed by 1 hour at 37°C, then were diluted in lysis buffer (100 mM potassium acetate, 30 mM HEPES-KOH at pH 7.4, 2mM magnesium acetate). Annealing was monitored by gel electrophoresis on an
30 agarose gel in TBE buffer and stained with ethidium bromide. The HeLa lysate was

prepared by a modified version of the Martinez protocol (Martinez J. *et al*, 2002, *Cell*, 110, 563-74). The assay comprises a reaction mixture containing 50% lysate [vol/vol], RNA target (10-50 pM final concentration), and 10% [vol/vol] lysis buffer containing siNA (100 nM final concentration). The reaction mixture also contains 200 μ M GTP, 2 mM ATP, 0.1 mM DTT, and 5 mM MgCl₂, 1 mM HEPES (pH 7.5). The reactions were pre-assembled on room temperature and preincubated at 30° C for 15 minutes before adding RNA target, then incubated at 30° C for an additional 160 minutes. Reactions were quenched with 2 volumes of 7M urea gel loading dye and snap frozen on dry ice. The specific RNA cleavage products generated by RNAi were separated on a dPAGE.

10 The percentage of cleavage was determined by PHOSPHOR IMAGER® (autoradiography) quantitation of bands representing intact control RNA or RNA from control reactions without siNA and the cleavage products generated by the assay. Several siNA constructs (sense/antisense compound numbers 36502/36518, 36503/36519, 36504/36520, 36505/36521, Table III) showed cleavage activity of

15 SNCA RNA in this system.

Example 7: Nucleic acid inhibition of PARK (e.g., SNCA) target RNA, *in vitro* cell culture experiments

siNA molecules targeted to the human PARK (e.g., SNCA) RNA are designed and synthesized as described above. These nucleic acid molecules can be tested for cleavage activity *in vivo*, for example, using the following procedure. The target sequences and the nucleotide location within the PARK (e.g., SNCA) RNA are given in Table II and

20 III.

Two formats are used to test the efficacy of siNAs targeting PARK (e.g., SNCA). First, the reagents are tested in cell culture using, for example, Cos-1, A375, A431, A549, or SK-N-SH cells, to determine the extent of RNA and protein inhibition. siNA reagents (e.g.; see Tables II and III) are selected against the PARK (e.g., SNCA) target as described herein. RNA inhibition is measured after delivery of these reagents by a suitable transfection agent to, for example, cultured Cos-1, A375, A431, A549, or SK-N-SH cells. Relative amounts of target RNA are measured versus actin using real-time

25 30 PCR monitoring of amplification (eg., ABI 7700 TAQMAN®). A comparison is made

- to a mixture of oligonucleotide sequences made to unrelated targets or to a randomized siNA control with the same overall length and chemistry, but randomly substituted at each position. Primary and secondary lead reagents are chosen for the target and optimization performed. After an optimal transfection agent concentration is chosen, a
- 5 RNA time-course of inhibition is performed with the lead siNA molecule. In addition, a cell-plating format can be used to determine RNA inhibition.

Delivery of siNA to Cells

- Cells (e.g., Cos-1, A375, A431, A549, or SK-N-SH cells) are seeded, for example, at 1×10^5 cells per well of a six-well dish in EGM-2 (BioWhittaker) the day before
- 10 transfection. siNA (final concentration, for example 20nM) and cationic lipid (e.g., final concentration $2 \mu\text{g/ml}$) are complexed in EGM basal media (Bio Whittaker) at 37°C for 30 minutes in polystyrene tubes. Following vortexing, the complexed siNA is added to each well and incubated for the times indicated. For initial optimization experiments, cells are seeded, for example, at 1×10^3 in 96 well plates and siNA complex added as
- 15 described. Efficiency of delivery of siNA to cells is determined using a fluorescent siNA complexed with lipid. Cells in 6-well dishes are incubated with siNA for 24 hours, rinsed with PBS and fixed in 2% paraformaldehyde for 15 minutes at room temperature. Uptake of siNA is visualized using a fluorescent microscope.

TAQMAN® (real-time PCR monitoring of amplification) and Lightcycler quantification of mRNA

- Total RNA is prepared from cells following siNA delivery, for example, using Qiagen RNA purification kits for 6-well or Rneasy extraction kits for 96-well assays. For TAQMAN® analysis (real-time PCR monitoring of amplification), dual-labeled probes are synthesized with the reporter dye, FAM or JOE, covalently linked at the 5'-end and
- 25 the quencher dye TAMRA conjugated to the 3'-end. One-step RT-PCR amplifications are performed on, for example, an ABI PRISM 7700 Sequence Detector using 50 μl reactions consisting of 10 μl total RNA, 100 nM forward primer, 900 nM reverse primer, 100 nM probe, 1X TaqMan PCR reaction buffer (PE-Applied Biosystems), 5.5 mM MgCl_2 , 300 μM each dATP, dCTP, dGTP, and dTTP, 10U RNase Inhibitor (Promega),
- 30 1.25U AMPLITAQ GOLD® (DNA polymerase) (PE-Applied Biosystems) and 10U M-

MLV Reverse Transcriptase (Promega). The thermal cycling conditions can consist of 30 minutes at 48°C, 10 minutes at 95°C, followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. Quantitation of mRNA levels is determined relative to standards generated from serially diluted total cellular RNA (300, 100, 33, 11 ng/rxn) and
5 normalizing to β -actin or GAPDH mRNA in parallel TAQMAN® reactions (real-time PCR monitoring of amplification). For each gene of interest an upper and lower primer and a fluorescently labeled probe are designed. Real time incorporation of SYBR Green I dye into a specific PCR product can be measured in glass capillary tubes using a lightcycler. A standard curve is generated for each primer pair using control cRNA.
10 Values are represented as relative expression to GAPDH in each sample.

Western blotting

Nuclear extracts can be prepared using a standard micro preparation technique (see for example Andrews and Faller, 1991, *Nucleic Acids Research*, 19, 2499). Protein extracts from supernatants are prepared, for example using TCA precipitation. An equal
15 volume of 20% TCA is added to the cell supernatant, incubated on ice for 1 hour and pelleted by centrifugation for 5 minutes. Pellets are washed in acetone, dried and resuspended in water. Cellular protein extracts are run on a 10% Bis-Tris NuPage (nuclear extracts) or 4-12% Tris-Glycine (supernatant extracts) polyacrylamide gel and transferred onto nitro-cellulose membranes. Non-specific binding can be blocked by
20 incubation, for example, with 5% non-fat milk for 1 hour followed by primary antibody for 16 hour at 4°C. Following washes, the secondary antibody is applied, for example (1:10,000 dilution) for 1 hour at room temperature and the signal detected with SuperSignal reagent (Pierce).

Example 8: RNAi mediated inhibition of PARK RNA expression, cell culture 25 experiment

siNA constructs (Table III) are tested for efficacy in reducing PARK (e.g., SNCA) RNA expression in, for example, neuro2a cells. Cells are plated approximately 24 hours before transfection in 96-well plates at 5,000-7,500 cells/well, 100 μ l/well, such that at the time of transfection cells are 70-90% confluent. For transfection, annealed siNAs are
30 mixed with the transfection reagent (Lipofectamine 2000, Invitrogen) in a volume of 50

- μl/well and incubated for 20 min. at room temperature. The siNA transfection mixtures are added to cells to give a final siNA concentration of 25 nM in a volume of 150 μl. Each siNA transfection mixture is added to 3 wells for triplicate siNA treatments. Cells are incubated at 37° for 24h in the continued presence of the siNA transfection mixture.
- 5 At 24h, RNA is prepared from each well of treated cells. The supernatants with the transfection mixtures are first removed and discarded, then the cells are lysed and RNA prepared from each well. Target gene expression following treatment is evaluated by RT-PCR for the target gene and for a control gene (36B4, an RNA polymerase subunit) for normalization. The triplicate data is averaged and the standard deviations determined
- 10 for each treatment. Normalized data are graphed and the percent reduction of target mRNA by active siNAs in comparison to their respective inverted control siNAs is determined.

Example 9: Animal Models useful to evaluate the down-regulation of PARK gene expression *in vivo*

- 15 Transgenic mice engrafted with the human alpha-synuclein gene develop a disorder characterized by slowness and paucity of movement (see Masliah *et al.*, 2000, *Science*, 287, 1265-1269) characterized by progressive accumulation of alpha-synuclein and ubiquitin immunoreactive inclusions in neurons in the neocortex, hippocampus, and substantia nigra, resulting in a disorder that resembles, to some extent, human Parkinson
- 20 disease. The mouse disorder is characterized by degeneration of dopamine cells associated with Lewy body-like inclusions. This model represents a relevant model to human Parkinson disease that can be used to evaluate nucleic acid molecules of the invention targeting PARK gene expression (e.g., SNCA RNA) for therapeutic and toxicological investigation using appropriate controls.

25 Example 11: Indications

The present body of knowledge in SNCA research indicates the need for methods to assay SNCA activity and for compounds that can regulate SNCA expression for research, diagnostic, and therapeutic use. As described herein, the nucleic acid molecules of the present invention can be used in assays to diagnose disease state related

of SNCA levels. In addition, the nucleic acid molecules can be used to treat disease state related to SNCA levels.

Particular conditions and disease states that can be associated with SNCA expression modulation include, but are not limited to neurodegenerative diseases, disorders, or conditions such as Parkinson's disease, Alzheimer's disease, dementia, and any other diseases or conditions that are related to or will respond to the levels of PARK1, PARK2, PARK7, and/or PARK5 in a cell or tissue, alone or in combination with other therapies (e.g. Levodopa; dopamine agonists; catechol-O-methyltransferase (COMT) inhibitors such as tolcapone and entacapone; surgical procedures such as thalamotomy, pallidotomy, and deep brain stimulation of the subthalamic nucleus, and transplantation and gene therapies).

Example 11: Diagnostic uses

The siNA molecules of the invention can be used in a variety of diagnostic applications, such as in the identification of molecular targets (e.g., RNA) in a variety of applications, for example, in clinical, industrial, environmental, agricultural and/or research settings. Such diagnostic use of siNA molecules involves utilizing reconstituted RNAi systems, for example, using cellular lysates or partially purified cellular lysates. siNA molecules of this invention can be used as diagnostic tools to examine genetic drift and mutations within diseased cells or to detect the presence of endogenous or exogenous, for example viral, RNA in a cell. The close relationship between siNA activity and the structure of the target RNA allows the detection of mutations in any region of the molecule, which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple siNA molecules described in this invention, one can map nucleotide changes, which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with siNA molecules can be used to inhibit gene expression and define the role of specified gene products in the progression of disease or infection. In this manner, other genetic targets can be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combination therapies (e.g., multiple siNA molecules targeted to different genes, siNA molecules coupled with known small molecule inhibitors, or intermittent treatment with combinations siNA

molecules and/or other chemical or biological molecules). Other *in vitro* uses of siNA molecules of this invention are well known in the art, and include detection of the presence of mRNAs associated with a disease, infection, or related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a
5 siNA using standard methodologies, for example, fluorescence resonance emission transfer (FRET).

In a specific example, siNA molecules that cleave only wild-type or mutant forms of the target RNA are used for the assay. The first siNA molecules (*i.e.*, those that cleave only wild-type forms of target RNA) are used to identify wild-type RNA present
10 in the sample and the second siNA molecules (*i.e.*, those that cleave only mutant forms of target RNA) are used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA are cleaved by both siNA molecules to demonstrate the relative siNA efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the
15 synthetic substrates also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus, each analysis requires two siNA molecules, two substrates and one unknown sample, which is combined into six reactions. The presence of cleavage products is determined using an RNase protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one
20 lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (*i.e.*, disease related or infection related) is adequate to establish risk. If probes of comparable specific activity are used for both transcripts,
25 then a qualitative comparison of RNA levels is adequate and decreases the cost of the initial diagnosis. Higher mutant form to wild-type ratios are correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. All references
30 cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The methods and compositions described herein as presently representative of preferred embodiments are exemplary and are not intended as
5 limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art, which are encompassed within the spirit of the invention, are defined by the scope of the claims.

It will be readily apparent to one skilled in the art that varying substitutions and modifications can be made to the invention disclosed herein without departing from the
10 scope and spirit of the invention. Thus, such additional embodiments are within the scope of the present invention and the following claims. The present invention teaches one skilled in the art to test various combinations and/or substitutions of chemical modifications described herein toward generating nucleic acid constructs with improved activity for mediating RNAi activity. Such improved activity can comprise improved
15 stability, improved bioavailability, and/or improved activation of cellular responses mediating RNAi. Therefore, the specific embodiments described herein are not limiting and one skilled in the art can readily appreciate that specific combinations of the modifications described herein can be tested without undue experimentation toward identifying siNA molecules with improved RNAi activity.

20 The invention illustratively described herein suitably can be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used
25 as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments, optional
30 features, modification and variation of the concepts herein disclosed may be resorted to

by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the description and the appended claims.

In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize
5 that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

Table I: PARK Accession Numbers

| | |
|----|---|
| 5 | NM_000345 Homo sapiens synuclein, alpha (non A4 component of amyloid precursor) (SNCA), transcript variant NACP140, mRNA gi 6806896 ref NM_000345.2 [6806896] |
| 10 | BC013293 Homo sapiens synuclein, alpha (non A4 component of amyloid precursor), transcript variant NACP140, mRNA (cDNA clone MGC:3484 IMAGE:3604532), complete |
| 15 | cds gi 33869957 gb BC013293.2 [33869957] |
| 20 | NM_007308 Homo sapiens synuclein, alpha (non A4 component of amyloid precursor) (SNCA), transcript variant NACP112, mRNA gi 6806897 ref NM_007308.1 [6806897] |
| 25 | NM_003085 Homo sapiens synuclein, beta (SNCB), mRNA gi 6466453 ref NM_003085.2 [6466453] |
| 30 | AY049786 Homo sapiens synuclein alpha (SNCA) mRNA, complete cds gi 16356656 gb AY049786.1 [16356656] |
| 35 | AF163864 Homo sapiens SNCA isoform (SNCA) gene, complete cds, alternatively spliced gi 11118351 gb AF163864.1 AF163864[11118351] |
| 40 | NM_007262 Homo sapiens Parkinson disease (autosomal recessive, early onset) 7 (PARK7), mRNA gi 34222306 ref NM_007262.3 [34222306] |
| 45 | BC022014 Homo sapiens Parkinson disease (autosomal recessive, juvenile) 2, parkin, mRNA (cDNA clone MGC:26491 IMAGE:4824892), complete cds |

- gi|34191069|gb|BC022014.2|[34191069]
- NT_007422
Homo sapiens chromosome 6 genomic contig
- 5 gi|29803241|ref|NT_007422.12|Hs6_7579[29803241]
- NM_013988
Homo sapiens Parkinson disease (autosomal recessive, juvenile) 2, parkin
- 10 (PARK2), transcript variant 3, mRNA
gi|7669539|ref|NM_013988.1|[7669539]
- NM_013987
Homo sapiens Parkinson disease (autosomal recessive, juvenile) 2, parkin
- 15 (PARK2), transcript variant 2, mRNA
gi|7669537|ref|NM_013987.1|[7669537]
- NM_004562
Homo sapiens Parkinson disease (autosomal recessive, juvenile) 2, parkin
- 20 (PARK2), transcript variant 1, mRNA
gi|4758883|ref|NM_004562.1|[4758883]
- 25 BC044227
Homo sapiens PARK2 co-regulated, mRNA (cDNA clone MGC:50733
IMAGE:5187302),
complete cds
- 30 gi|28279820|gb|BC044227.1|[28279820]
- BC030642
Homo sapiens PARK2 co-regulated, mRNA (cDNA clone MGC:26712
- 35 IMAGE:4823539),
complete cds
gi|34190122|gb|BC030642.2|[34190122]
- NM_152410
Homo sapiens PARK2 co-regulated (PACRG), mRNA
- 40 gi|22748868|ref|NM_152410.1|[22748868]
- NT_021937
Homo sapiens chromosome 1 genomic contig
- 45 gi|37539904|ref|NT_021937.16|Hs1_22093[37539904]

- NM_007262
Homo sapiens Parkinson disease (autosomal recessive, early onset) 7 (PARK7),
mRNA
gi|34222306|ref|NM_007262.3|[34222306]
- 5
- NM_013988
Homo sapiens Parkinson disease (autosomal recessive, juvenile) 2, parkin
(PARK2), transcript variant 3, mRNA
gi|7669539|ref|NM_013988.1|[7669539]
- 10
- NM_013987
Homo sapiens Parkinson disease (autosomal recessive, juvenile) 2, parkin
(PARK2), transcript variant 2, mRNA
gi|7669537|ref|NM_013987.1|[7669537]
- 15
- NM_004562
Homo sapiens Parkinson disease (autosomal recessive, juvenile) 2, parkin
(PARK2), transcript variant 1, mRNA
gi|4758883|ref|NM_004562.1|[4758883]
- 20
- BC008188
Homo sapiens Parkinson disease (autosomal recessive, early onset) 7, mRNA (cDNA
clone MGC:5243 IMAGE:2901102), complete cds
gi|34193707|gb|BC008188.2|[34193707]
- 25
- NM_004181
Homo sapiens ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase)
(UCHL1), mRNA
gi|34147658|ref|NM_004181.3|[34147658]
- 30
- BC000332
Homo sapiens ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase),
mRNA (cDNA clone MGC:8524 IMAGE:2822541), complete cds
gi|33875314|gb|BC000332.2|[33875314]
- 35
- BC005117
Homo sapiens ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase),
mRNA (cDNA clone MGC:3601 IMAGE:2823559), complete cds
gi|13477286|gb|BC005117.1|[13477286]
- 40
- AF053072
Homo sapiens GABA subunit A receptor alpha 6 precursor, gene, partial cds
gi|4405812|gb|AF053072.1|AF053072[4405812]
- 45

(400/163)

Table II: SNCA siNA and Target Sequences

SNCA NM_000345.2

| Pos | Target Seq | Seq ID | UPos | Upper seq | Seq ID | LPos | Lower seq | Seq ID |
|-----|----------------------|--------|------|----------------------|--------|------|-----------------------|--------|
| 3 | AGUGGCCAUUCGACGACAG | 1 | 3 | AGUGGCCAUUCGACGACAG | 1 | 21 | CUGUCGUGAAUUGGCCACU | 87 |
| 21 | GUGUGUGUAAAGGAUUC | 2 | 21 | GUGUGUGUAAAGGAUUC | 2 | 39 | GAAUCCUUUACACCCAC | 88 |
| 39 | CAUUAAGCCAUUGGAUUAU | 3 | 39 | CAUUAAGCCAUUGGAUUAU | 3 | 57 | AAUACAUCUAGGCUAAUG | 89 |
| 57 | UCAUGAAAGGACUUAUCAA | 4 | 57 | UCAUGAAAGGACUUAUCAA | 4 | 75 | UUUGAAAGUCCUUUCAUGA | 90 |
| 75 | AGCCCAAGGAGGAGUUGU | 5 | 75 | AGCCCAAGGAGGAGUUGU | 5 | 93 | ACAACUCCUCCUUGGCCU | 91 |
| 93 | UGGCUUGCUUGAGAAAC | 6 | 93 | UGGCUUGCUUGAGAAAC | 6 | 111 | GUUUUCUCAGCAGCAGCCA | 92 |
| 111 | CCAAACAGGGUGUGGCAGA | 7 | 111 | CCAAACAGGGUGUGGCAGA | 7 | 129 | UCUGCCACACCCUUGUUGG | 93 |
| 129 | AAGCAGCAGGAAAGACAAA | 8 | 129 | AAGCAGCAGGAAAGACAAA | 8 | 147 | UUUGUUUUCCUGUCUU | 94 |
| 147 | AAGAGGGUUCUCUAUGU | 9 | 147 | AAGAGGGUUCUCUAUGU | 9 | 165 | ACAUAGAGAACCCUUCU | 95 |
| 165 | UAGGCUCCAAACCAAGGA | 10 | 165 | UAGGCUCCAAACCAAGGA | 10 | 183 | UCCUUGUUUUUGGAGCUA | 96 |
| 183 | AGGAGUGGUGCAUGGUGU | 11 | 183 | AGGAGUGGUGCAUGGUGU | 11 | 201 | ACACCAUGCAGCAGCUCGU | 97 |
| 201 | UGGCAACAGUGGCUGAGAA | 12 | 201 | UGGCAACAGUGGCUGAGAA | 12 | 219 | UUCAGCCACUGUUGCCA | 98 |
| 219 | AGACCAAGAGCAAGUGAC | 13 | 219 | AGACCAAGAGCAAGUGAC | 13 | 237 | GUCACUUGCUCUUUGGUCU | 99 |
| 237 | CAAAUUGUGGAGGAGCAGU | 14 | 237 | CAAAUUGUGGAGGAGCAGU | 14 | 255 | ACUGUCCUCCAACAUAUUG | 100 |
| 255 | UGGUGACGGGUGUGACAGC | 15 | 255 | UGGUGACGGGUGUGACAGC | 15 | 273 | GCUGUCACACCCGUCACCA | 101 |
| 273 | CAGUAGCCCAAGAGACAGU | 16 | 273 | CAGUAGCCCAAGAGACAGU | 16 | 291 | ACUGUUCUUGGGGCUACUG | 102 |
| 291 | UGGAGGGAGCAGGGAGCAU | 17 | 291 | UGGAGGGAGCAGGGAGCAU | 17 | 309 | AUGCUCUUGCUCUCCUCCA | 103 |
| 309 | UUGCAGCAGCCACUUGGCUU | 18 | 309 | UUGCAGCAGCCACUUGGCUU | 18 | 327 | AAGCAGUGGCGUCGCAA | 104 |
| 327 | UUUCUAAAAGGACCAAGU | 19 | 327 | UUUCUAAAAGGACCAAGU | 19 | 345 | AACUGUCCUUUUUUGACAA | 105 |
| 345 | UGGCAAGAAUGAAGAGG | 20 | 345 | UGGCAAGAAUGAAGAGG | 20 | 363 | CCUUCUUAUUCUUGCCCA | 106 |
| 363 | GAGCCCCACAGGAAGGAU | 21 | 363 | GAGCCCCACAGGAAGGAU | 21 | 381 | AUUCUUCUUGUGGGGCU | 107 |
| 381 | UUCUGGAAGAUUGCCUGU | 22 | 381 | UUCUGGAAGAUUGCCUGU | 22 | 399 | ACAGGCAUAUUCUCCAGAA | 108 |
| 399 | UGGAUCCUGACAAUGAGGC | 23 | 399 | UGGAUCCUGACAAUGAGGC | 23 | 417 | GCCUUAUUGCAGGGAUCCA | 109 |
| 417 | CUUAUGAAUUGCCUUCUGA | 24 | 417 | CUUAUGAAUUGCCUUCUGA | 24 | 435 | UCAGAAGGCAUUUCAUAAAG | 110 |
| 435 | AGGAAGGUAUCAAGACUA | 25 | 435 | AGGAAGGUAUCAAGACUA | 25 | 453 | UAGUUAUUAUACCCUUCU | 111 |
| 453 | ACGAACCUAGAGCCUAAGA | 26 | 453 | ACGAACCUAGAGCCUAAGA | 26 | 471 | UCUUAAGGCUUACAGGUUCGU | 112 |
| 471 | AAUAUUCUUGCUCUCCAGU | 27 | 471 | AAUAUUCUUGCUCUCCAGU | 27 | 489 | ACUGGAGCAAAAGAUUUU | 113 |
| 489 | UUUCUUGAGAUUCUGUGAC | 28 | 489 | UUUCUUGAGAUUCUGUGAC | 28 | 507 | GUCAGCAUUCUCAAGAAA | 114 |
| 507 | CAGAUGUCCAUCCUGUAC | 29 | 507 | CAGAUGUCCAUCCUGUAC | 29 | 525 | GUACAGGAUGGAACAUCUG | 115 |
| 525 | CAAGUGCUCAGUCCAAUG | 30 | 525 | CAAGUGCUCAGUCCAAUG | 30 | 543 | CAUUGGAACUGAGCAGUUG | 116 |
| 543 | GUGCCCAGUCAUGACAUUU | 31 | 543 | GUGCCCAGUCAUGACAUUU | 31 | 561 | AAAUUGCAUGACUGGGCAC | 117 |

(400/163)

| | | | | | | | | |
|------|----------------------|----|------|----------------------|----|------|----------------------|-----|
| 561 | UCUCAAGUUUUUACAGUG | 32 | 561 | UCUCAAGUUUUUACAGUG | 32 | 579 | CACUGUAAAAACUUUGAGA | 118 |
| 579 | GUUUCUCGAAGUCUUCU | 33 | 579 | GUUUCUCGAAGUCUUCU | 33 | 597 | AUGGAAGACUUCGAGAUAC | 119 |
| 597 | UCAGCAGUGUUAAGUAU | 34 | 597 | UCAGCAGUGUUAAGUAU | 34 | 615 | AUACUUAACACUGCUGA | 120 |
| 615 | UCUGUACCUCCCCACUC | 35 | 615 | UCUGUACCUCCCCACUC | 35 | 633 | GAGUGGGGCGAGGUACAGA | 121 |
| 633 | CAGCAUUUCGGUGCUUCC | 36 | 633 | CAGCAUUUCGGUGCUUCC | 36 | 651 | GGGAAGCACCGAAUUGCUG | 122 |
| 651 | CUUUCACUGAAGUGAUAC | 37 | 651 | CUUUCACUGAAGUGAUAC | 37 | 669 | GUUUCACUUCAGUGAAAG | 123 |
| 669 | CAUGGUGAGCAGGGUCUUG | 38 | 669 | CAUGGUGAGCAGGGUCUUG | 38 | 687 | CAAGACCCUGCUACCAUG | 124 |
| 687 | GUGUGCUGUGGAUUUUGUG | 39 | 687 | GUGUGCUGUGGAUUUUGUG | 39 | 705 | CACAAAUCACACAGCACAC | 125 |
| 705 | GGCUUCAUCUACGAGUU | 40 | 705 | GGCUUCAUCUACGAGUU | 40 | 723 | AACAUCGUAGAUUGAAGCC | 126 |
| 723 | UAAAACAAUUAAAAACAC | 41 | 723 | UAAAACAAUUAAAAACAC | 41 | 741 | GUGUUUUUAAUUUGUUUA | 127 |
| 741 | CCUAAAGUGACUACCAUUA | 42 | 741 | CCUAAAGUGACUACCAUUA | 42 | 759 | UAAGUGGUGACUCUUAGG | 128 |
| 759 | AUUUCUAAUCCUCACUUA | 43 | 759 | AUUUCUAAUCCUCACUUA | 43 | 777 | AUAGUGAGGUUUUAGAAU | 129 |
| 777 | UUUUUUUGUGCUGUUGUU | 44 | 777 | UUUUUUUGUGCUGUUGUU | 44 | 795 | AACAACGACCAACAAAAA | 130 |
| 795 | UCAGAAUUUUAAGUUAU | 45 | 795 | UCAGAAUUUUAAGUUAU | 45 | 813 | AAUCACUAAACAGUUCUGA | 131 |
| 813 | UUGCUAUCAUUAUUAUA | 46 | 813 | UUGCUAUCAUUAUUAUA | 46 | 831 | UUUAUAUAUUAUUAAGCAA | 132 |
| 831 | AGAUUUUUAAGGUGUCUUU | 47 | 831 | AGAUUUUUAAGGUGUCUUU | 47 | 849 | AAAAGACCCUAAAAUCU | 133 |
| 849 | UAAUGAUACUGUCUAAAGAA | 48 | 849 | UAAUGAUACUGUCUAAAGAA | 48 | 867 | UUCUUAGACAGUAUCAUUA | 134 |
| 867 | AUAAUGACGUUAUUGUGAAA | 49 | 867 | AUAAUGACGUUAUUGUGAAA | 49 | 885 | UUUCACAAUACGUAUUAU | 135 |
| 885 | AUUUGUUAUAUAUAUAU | 50 | 885 | AUUUGUUAUAUAUAUAU | 50 | 903 | AUUAUAUAUAUAUAACAAU | 136 |
| 903 | UACUUAUUAUAUUGAGC | 51 | 903 | UACUUAUUAUAUUGAGC | 51 | 921 | GCUCACAUUUUUUUAAGUA | 137 |
| 921 | CAUGAAACUUAUGCACCUAU | 52 | 921 | CAUGAAACUUAUGCACCUAU | 52 | 939 | AUAGGUGCAUUAUUUCAUG | 138 |
| 939 | UAAUAUUAUAUAUUGAAU | 53 | 939 | UAAUAUUAUAUAUUGAAU | 53 | 957 | AUUUCAUUAUUUAGUAUUA | 139 |
| 957 | UUUUACCAUUUUGCGAUGU | 54 | 957 | UUUUACCAUUUUGCGAUGU | 54 | 975 | ACAUCGCAAAUUGGUAAA | 140 |
| 975 | UGUUUUUAUUCUUGUGUU | 55 | 975 | UGUUUUUAUUCUUGUGUU | 55 | 993 | AACACAAGUGAAUUAACA | 141 |
| 993 | UUGUAUAUAUAUUGGUGAGA | 56 | 993 | UUGUAUAUAUAUUGGUGAGA | 56 | 1011 | UCUCACCAUUUAUAUACAA | 142 |
| 1011 | AUUUAAAAUAAACGUUAU | 57 | 1011 | AUUUAAAAUAAACGUUAU | 57 | 1029 | AUAACGUUUUUUUUUUAUU | 143 |
| 1029 | UCUCAUUGCAAAAUUAUUU | 58 | 1029 | UCUCAUUGCAAAAUUAUUU | 58 | 1047 | AAUAUUUUUGCAUUGAGA | 144 |
| 1047 | UUAUUUUUAUCCCAUCUCA | 59 | 1047 | UUAUUUUUAUCCCAUCUCA | 59 | 1065 | UGAGAUUGGGAUAAAAUAA | 145 |
| 1065 | ACUUUUAUAUAAAAUUA | 60 | 1065 | ACUUUUAUAUAAAAUUA | 60 | 1083 | UGAUUUUUUAUUUAAGU | 146 |
| 1083 | AUGCUUAUAAGCAACAUGA | 61 | 1083 | AUGCUUAUAAGCAACAUGA | 61 | 1101 | UCAUGUUGCUUAUAAGCAU | 147 |
| 1101 | AUUUAAGAACUGACACAAA | 62 | 1101 | AUUUAAGAACUGACACAAA | 62 | 1119 | UUUGUGCAGUUCUUUAUU | 148 |
| 1119 | AGGACAAAAUAUAUAAAGUU | 63 | 1119 | AGGACAAAAUAUAUAAAGUU | 63 | 1137 | AACUUUAUUUUUUGUCCU | 149 |
| 1137 | UAUUUAUAGCCAUUUUGAAG | 64 | 1137 | UAUUUAUAGCCAUUUUGAAG | 64 | 1155 | CUUCAAAUUGGUUAUUAUA | 150 |
| 1155 | GAAGGAGAAUUUUUAGAA | 65 | 1155 | GAAGGAGAAUUUUUAGAA | 65 | 1173 | CUUCUAAAAUCCUCCUUC | 151 |
| 1173 | GAGGUAGAGAAUUGGAAC | 66 | 1173 | GAGGUAGAGAAUUGGAAC | 66 | 1191 | GUUCCAUUUUCUCUACCCUC | 152 |
| 1191 | CAUUAACCCUACACUCGGA | 67 | 1191 | CAUUAACCCUACACUCGGA | 67 | 1209 | UCCGAGUGUAGGGUUAUUG | 153 |

(400/163)

| | | | | | | | | |
|------|---------------------|----|------|---------------------|----|------|----------------------|-----|
| 1209 | AAUUCUUUUAAGCAACACU | 68 | 1209 | AAUUCUUUUAAGCAACACU | 68 | 1227 | AGUUUUGUUUUAAGCAAUU | 154 |
| 1227 | UGCCAGAGUGUGUUUUUGG | 69 | 1227 | UGCCAGAGUGUGUUUUUGG | 69 | 1245 | CCAAAACACAUUCUGGCA | 155 |
| 1245 | GUAUGCAGUGUUUUUUAA | 70 | 1245 | GUAUGCAGUGUUUUUUAA | 70 | 1263 | UUAAGGAACACAGUGCAUAC | 156 |
| 1263 | AGUGGCUUGUUUUUUUU | 71 | 1263 | AGUGGCUUGUUUUUUUU | 71 | 1281 | AUAUUUUAUACACAGCCACU | 157 |
| 1281 | UUGAAAGUGGGUGUUUGAA | 72 | 1281 | UUGAAAGUGGGUGUUUGAA | 72 | 1299 | UUAACACCCACAUUUUCAA | 158 |
| 1299 | AGACCCCAACUUAUUUGU | 73 | 1299 | AGACCCCAACUUAUUUGU | 73 | 1317 | ACAUAAGUAGUUGGGUUCU | 159 |
| 1317 | UAGAGUGGCUUAUUUCUCC | 74 | 1317 | UAGAGUGGCUUAUUUCUCC | 74 | 1335 | GGAGAAUUAAGACACUCUA | 160 |
| 1335 | CCUCAAUCCUGUCAUUGU | 75 | 1335 | CCUCAAUCCUGUCAUUGU | 75 | 1353 | ACAUUGACAGGAUUGAAGG | 161 |
| 1353 | UUUGCUUAUGUAUUUUUGG | 76 | 1353 | UUUGCUUAUGUAUUUUUGG | 76 | 1371 | CCAAAAUACAUAUAAAGCAA | 162 |
| 1371 | GGGAACUGUUUUUGAUGU | 77 | 1371 | GGGAACUGUUUUUGAUGU | 77 | 1389 | ACAUCAAAACACAGUUCUCC | 163 |
| 1389 | UGUAUGUGUUUAUUUUUGU | 78 | 1389 | UGUAUGUGUUUAUUUUUGU | 78 | 1407 | ACAUUUAUAAACACAUACA | 164 |
| 1407 | UUUAACAUUUUUAUUUGAG | 79 | 1407 | UUUAACAUUUUUAUUUGAG | 79 | 1425 | CUCAUUUAAAAUUGUAUA | 165 |
| 1425 | GCCUUUUUAUAAACAUAUU | 80 | 1425 | GCCUUUUUAUAAACAUAUU | 80 | 1443 | AUAUUGUUUAUAAUAAAGGC | 166 |
| 1443 | UUGUUUAUUUUUGUCUGAA | 81 | 1443 | UUGUUUAUUUUUGUCUGAA | 81 | 1461 | UUCGAGACAAAAUUAACAA | 167 |
| 1461 | AAUAUUUUUAUUAUAAAA | 82 | 1461 | AAUAUUUUUAUUAUAAAA | 82 | 1479 | UUUUAACUAAAAUUUUUU | 168 |
| 1479 | AUCUAUUUUUGUCUGAUUU | 83 | 1479 | AUCUAUUUUUGUCUGAUUU | 83 | 1497 | AUAUCAGACAAAAUAGAU | 169 |
| 1497 | UGGUGUGAAUGCUGUACCU | 84 | 1497 | UGGUGUGAAUGCUGUACCU | 84 | 1515 | AGGUACAGCAUUCACACCA | 170 |
| 1515 | UUUCUGACAAUAAUAUA | 85 | 1515 | UUUCUGACAAUAAUAUA | 85 | 1533 | UAUUUUUUUUUUGUCAGAAA | 171 |
| 1523 | AAUAAUUAUUUUGACCA | 86 | 1523 | AAUAAUUAUUUUGACCA | 86 | 1541 | UGGUGGAAUUAUUUUUUUU | 172 |

SNCA NM_000345.2 mutants

| Pos | Target Seq | Seq ID | UPos | Upper seq | Seq ID | LPos | Lower seq | Seq ID |
|-----|----------------------|--------|------|----------------------|--------|------|----------------------|--------|
| 116 | CAGGGUGUGGCAGAGCAC | 173 | 116 | CAGGGUGUGGCAGAGCAC | 173 | 134 | GUGCUUCUGCCACACCCUG | 211 |
| 117 | AGGGUGUGGCAGAGCAC | 174 | 117 | AGGGUGUGGCAGAGCAC | 174 | 135 | GGUGCUUCUGCCACACCCU | 212 |
| 118 | GGUGUGGCAGAGCACCA | 175 | 118 | GGUGUGGCAGAGCACCA | 175 | 136 | UGGUGCUUCUGCCACACCC | 213 |
| 119 | GGUGUGGCAGAGCACCCAG | 176 | 119 | GGUGUGGCAGAGCACCCAG | 176 | 137 | CUGGUGCUUCUGCCACACCC | 214 |
| 120 | GUGUGGCAGAGCACCCAGG | 177 | 120 | GUGUGGCAGAGCACCCAGG | 177 | 138 | CCUGGUGCUUCUGCCACAC | 215 |
| 121 | UGUGGCAGAGCACCCAGGA | 178 | 121 | UGUGGCAGAGCACCCAGGA | 178 | 139 | UCCUGGUGCUUCUGCCACA | 216 |
| 122 | GUGGCAGAGCACCCAGGAA | 179 | 122 | GUGGCAGAGCACCCAGGAA | 179 | 140 | UUCUGGUGCUUCUGCCAC | 217 |
| 123 | UGGCAGAGCACCCAGGAAA | 180 | 123 | UGGCAGAGCACCCAGGAAA | 180 | 141 | UUUCCUGGUGCUUCUGCCA | 218 |
| 124 | GGCAGAGCACCCAGGAAAG | 181 | 124 | GGCAGAGCACCCAGGAAAG | 181 | 142 | CUUCCUGGUGCUUCUGCC | 219 |
| 125 | GCAGAGCACCCAGGAAAGA | 182 | 125 | GCAGAGCACCCAGGAAAGA | 182 | 143 | UCUUCCUGGUGCUUCUGC | 220 |
| 126 | CAGAAGCACCCAGGAAAGAC | 183 | 126 | CAGAAGCACCCAGGAAAGAC | 183 | 144 | GUCUUCCUGGUGCUUCUG | 221 |
| 127 | AGAAGCACCCAGGAAAGACA | 184 | 127 | AGAAGCACCCAGGAAAGACA | 184 | 145 | UUCUUCCUGGUGCUUCU | 222 |
| 128 | GAAGCACCCAGGAAAGACAA | 185 | 128 | GAAGCACCCAGGAAAGACAA | 185 | 146 | UUGUCUUCCUGGUGCUUC | 223 |

(400/163)

| | | | | | | | | |
|-----|---------------------|-----|---------------------|-----|-----|-----|---------------------|-----|
| 129 | AAGCACCAGGAAAGACAAA | 186 | AAGCACCAGGAAAGACAAA | 129 | 186 | 147 | UUUGUCUUUCCUGGUCUU | 224 |
| 130 | AGCACACAGGAAAGACAAA | 187 | AGCACACAGGAAAGACAAA | 130 | 187 | 148 | UUUGUCUUUCCUGGUCU | 225 |
| 131 | GCACAGGAAAGACAAAAG | 188 | GCACAGGAAAGACAAAAG | 131 | 188 | 149 | UUUGUCUUUCCUGGUGC | 226 |
| 132 | CACCAGGAAAGACAAAAGA | 189 | CACCAGGAAAGACAAAAGA | 132 | 189 | 150 | UCUUUUGUCUUUCCUGGUG | 227 |
| 133 | ACCAGGAAAGACAAAAGAG | 190 | ACCAGGAAAGACAAAAGAG | 133 | 190 | 151 | CUCUUUUGUCUUUCCUGGU | 228 |
| 134 | CCAGGAAAGACAAAAGAGG | 191 | CCAGGAAAGACAAAAGAGG | 134 | 191 | 152 | CCUCUUUUGUCUUUCCUGG | 229 |
| 191 | GUGCAUGGUGGCAACAA | 192 | GUGCAUGGUGGCAACAA | 191 | 192 | 209 | UUUGUCCACACCAUGCAC | 230 |
| 192 | UGCAUGGUGGCAACAAU | 193 | UGCAUGGUGGCAACAAU | 192 | 193 | 210 | AUUGUUGCCACACCAUGCA | 231 |
| 193 | GCAUGGUGGCAACAAUG | 194 | GCAUGGUGGCAACAAUG | 193 | 194 | 211 | CAUUGUUGCCACACCAUGC | 232 |
| 194 | CAUGGUGGCAACAAUGG | 195 | CAUGGUGGCAACAAUGG | 194 | 195 | 212 | CCAUGUUGCCACACCAUG | 233 |
| 195 | AUGGUGGCAACAAUGGC | 196 | AUGGUGGCAACAAUGGC | 195 | 196 | 213 | GCCAUGUUGCCACACCAU | 234 |
| 196 | UGGUGGCAACAAUGGCU | 197 | UGGUGGCAACAAUGGCU | 196 | 197 | 214 | AGCCAUGUUGCCACACCA | 235 |
| 197 | GGUGGCAACAAUGGCU | 198 | GGUGGCAACAAUGGCU | 197 | 198 | 215 | CAGCCAUGUUGCCACACC | 236 |
| 198 | GUGUGGCAACAAUGGCU | 199 | GUGUGGCAACAAUGGCU | 198 | 199 | 216 | UCAGCCAUGUUGCCACAC | 237 |
| 199 | UGUGGCAACAAUGGCU | 200 | UGUGGCAACAAUGGCU | 199 | 200 | 217 | CUCAGCCAUGUUGCCACA | 238 |
| 200 | GUGGCAACAAUGGCU | 201 | GUGGCAACAAUGGCU | 200 | 201 | 218 | UCUCAGCCAUGUUGCCAC | 239 |
| 201 | UGGCAACAAUGGCU | 202 | UGGCAACAAUGGCU | 201 | 202 | 219 | UUCUCAGCCAUGUUGCCA | 240 |
| 202 | GGCAACAAUGGCU | 203 | GGCAACAAUGGCU | 202 | 203 | 220 | CUUCUCAGCCAUGUUGCC | 241 |
| 203 | GCAACAAUGGCU | 204 | GCAACAAUGGCU | 203 | 204 | 221 | UCUUCUCAGCCAUGUUGC | 242 |
| 204 | CAACAAUGGCU | 205 | CAACAAUGGCU | 204 | 205 | 222 | GUCUUCUCAGCCAUGUUG | 243 |
| 205 | AACAUGGCU | 206 | AACAUGGCU | 205 | 206 | 223 | GGUCUUCUCAGCCAUGUU | 244 |
| 206 | ACAUGGCU | 207 | ACAUGGCU | 206 | 207 | 224 | UGGUCUUCUCAGCCAUGU | 245 |
| 207 | CAAUGGCU | 208 | CAAUGGCU | 207 | 208 | 225 | UUGUCUUCUCAGCCAUG | 246 |
| 208 | AUUGGCU | 209 | AUUGGCU | 208 | 209 | 226 | UUUGGCUUCUCAGCCAU | 247 |
| 209 | AUGGCU | 210 | AUGGCU | 209 | 210 | 227 | CUUUGGCUUCUCAGCCA | 248 |

The 3'-ends of the Upper sequence and the Lower sequence of the siNA construct can include an overhang sequence, for example about 1, 2, 3, or 4 nucleotides in length, preferably 2 nucleotides in length, wherein the overhang sequence of the lower sequence is optionally complementary to a portion of the target sequence. The overhang can comprise the general structure NN or NsN, where N stands for any nucleotide (e.g., thymidine) and s stands for phosphorothioate or other internucleotide linkage as described herein (e.g. internucleotide linkage having Formula I). The upper sequence is also referred to as the sense strand, whereas the lower sequence is also referred to as the antisense strand. The upper and lower sequences in the Table can further comprise a chemical modification having Formulae I-VII or any combination thereof (see for example chemical modifications as shown in Table IV herein).

(400/163)

Table III: SNCA Synthetic Modified siNA constructs

| Target Pos | Target Seq. | SeqID | Compound# | Aliases | Sequence | SeqID |
|------------|-------------------------|-------|-----------|--|---------------------------|-------|
| 389 | GAUAUGCCUUGGUAUCCUGACAA | 249 | | SNCA:391U21 siNA sense | UAUGCCUUGGUAUCCUGACTT | 253 |
| 422 | GAUAUGCCUUCUGAGGAAGGGUA | 250 | | SNCA:424U21 siNA sense | AAUGCCUUCUGAGGAAGGGTT | 254 |
| 673 | GUAGCAGGGUCUUGUGUGCUGU | 251 | | SNCA:675U21 siNA sense | AGCAGGGUCUUGUGUGCUTT | 255 |
| 1335 | CCUCAAUCCUGUCAAUUGUUUGC | 252 | | SNCA:1337U21 siNA sense | UUCAUCCUGUCAAUUGUUUTT | 256 |
| 389 | GAUAUGCCUUGGUAUCCUGACAA | 249 | | SNCA:409L21 siNA (391C) antisense | GUCAGGAUCCACAGGCAUATT | 257 |
| 422 | GAUAUGCCUUCUGAGGAAGGGUA | 250 | | SNCA:442L21 siNA (424C) antisense | CCUUCUCCUCAGGAAGGCAUUTT | 258 |
| 673 | GUAGCAGGGUCUUGUGUGCUGU | 251 | | SNCA:693L21 siNA (675C) antisense | AGCACACAAAAGACCCUGCUTT | 259 |
| 1335 | CCUCAAUCCUGUCAAUUGUUUGC | 252 | | SNCA:1355L21 siNA (1337C) antisense | AAACAUUGACAGGAUUGAATT | 260 |
| 389 | GAUAUGCCUUGGUAUCCUGACAA | 249 | | SNCA:391U21 siNA stab04 sense | B uAuGccuUGGUAuccuGAcTT B | 261 |
| 422 | GAUAUGCCUUCUGAGGAAGGGUA | 250 | | SNCA:424U21 siNA stab04 sense | B AAUGccuUGGUAuccuGAcTT B | 262 |
| 673 | GUAGCAGGGUCUUGUGUGCUGU | 251 | | SNCA:675U21 siNA stab04 sense | B AGcAGGGUcucuUGuGcuTT B | 263 |
| 1335 | CCUCAAUCCUGUCAAUUGUUUGC | 252 | | SNCA:1337U21 siNA stab04 sense | B uuCAuuccuGucAAuGuuuTT B | 264 |
| 389 | GAUAUGCCUUGGUAUCCUGACAA | 249 | | SNCA:409L21 siNA (391C) stab05 antisense | GucAGGAuccAcAGGcAuATsT | 265 |
| 422 | GAUAUGCCUUCUGAGGAAGGGUA | 250 | | SNCA:442L21 siNA (424C) stab05 antisense | cccuuccucAGAAAGGcAuTTsT | 266 |
| 673 | GUAGCAGGGUCUUGUGUGCUGU | 251 | | SNCA:693L21 siNA (675C) stab05 antisense | AGcAcAcAAAGAcCCcGcuTsT | 267 |
| 1335 | CCUCAAUCCUGUCAAUUGUUUGC | 252 | | SNCA:1355L21 siNA (1337C) stab05 antisense | AAAcAuUGAcAGGAuuGAATsT | 268 |
| 389 | GAUAUGCCUUGGUAUCCUGACAA | 249 | | SNCA:391U21 siNA stab07 sense | B uAuGccuUGGUAuccuGAcTT B | 269 |
| 422 | GAUAUGCCUUCUGAGGAAGGGUA | 250 | | SNCA:424U21 siNA stab07 sense | B AAUGccuUGGUAuccuGAcTT B | 270 |
| 673 | GUAGCAGGGUCUUGUGUGCUGU | 251 | | SNCA:675U21 siNA stab07 sense | B AGcAGGGUcucuUGuGcuTT B | 271 |
| 1335 | CCUCAAUCCUGUCAAUUGUUUGC | 252 | | SNCA:1337U21 siNA stab07 sense | B uuCAuuccuGucAAuGuuuTT B | 272 |
| 389 | GAUAUGCCUUGGUAUCCUGACAA | 249 | | SNCA:409L21 siNA (391C) stab11 antisense | GucAGGAuccAcAGGcAuATsT | 273 |
| 422 | GAUAUGCCUUCUGAGGAAGGGUA | 250 | | SNCA:442L21 siNA (424C) stab11 antisense | cccuuccucAGAAAGGcAuTTsT | 274 |
| 673 | GUAGCAGGGUCUUGUGUGCUGU | 251 | | SNCA:693L21 siNA (675C) stab11 antisense | AGcAcAcAAAGAcCCcGcuTsT | 275 |
| 1335 | CCUCAAUCCUGUCAAUUGUUUGC | 252 | | SNCA:1355L21 siNA (1337C) stab11 antisense | AAAcAuUGAcAGGAuuGAATsT | 276 |

(400/163)

| | | | | | | |
|------|-------------------------|-----|--|--|-----------------------------|-----|
| 389 | GAUAGCCUUGUGGAUCCUGACAA | 249 | | SNCA:391U21 siNA stab18 sense | B uAUGccuGUGGAuccuGAcTT B | 277 |
| 422 | GAAUAGCCUUCUGAGGAAGGGUA | 250 | | SNCA:424U21 siNA stab18 sense | B AAUGccuucuuGAGGAAGGGTT B | 278 |
| 673 | GUAGCAGGGUUCUUGUGUGCUGU | 251 | | SNCA:675U21 siNA stab18 sense | B AGcAGGGGucuuuuGuGuGcuTT B | 279 |
| 1335 | CCUUCAAUCCUGUCAUUGUUGC | 252 | | SNCA:1337U21 siNA stab18 sense | B uucaAuccuGucAAuGuuuTT B | 280 |
| 389 | GAUAGCCUUGUGGAUCCUGACAA | 249 | | SNCA:409L21 siNA (391C) stab08 antisense | GucAGGAuccAcAGGcAuATsT | 281 |
| 422 | GAAUAGCCUUCUGAGGAAGGGUA | 250 | | SNCA:442L21 siNA (424C) stab08 antisense | cccuuccucAGAAAGGcAuTsT | 282 |
| 673 | GUAGCAGGGUUCUUGUGUGCUGU | 251 | | SNCA:693L21 siNA (675C) stab08 antisense | AGcAcAAAGAcuccuGcuTsT | 283 |
| 1335 | CCUUCAAUCCUGUCAUUGUUGC | 252 | | SNCA:1355L21 siNA (1337C) stab08 antisense | AAcAuuuGAcAGGAuuGAATsT | 284 |
| 389 | GAUAGCCUUGUGGAUCCUGACAA | 249 | | SNCA:391U21 siNA stab09 sense | B UAUGCCUGUGGAUCCUGACTT B | 285 |
| 422 | GAAUAGCCUUCUGAGGAAGGGUA | 250 | | SNCA:424U21 siNA stab09 sense | B AAUGCCUUCUGAGGAAGGGTT B | 286 |
| 673 | GUAGCAGGGUUCUUGUGUGCUGU | 251 | | SNCA:675U21 siNA stab09 sense | B AGCAGGGUUCUUGUGUGCUTT B | 287 |
| 1335 | CCUUCAAUCCUGUCAUUGUUGC | 252 | | SNCA:1337U21 siNA stab09 sense | B UUCAUCCUGUCAUUGUUTT B | 288 |
| 389 | GAUAGCCUUGUGGAUCCUGACAA | 249 | | SNCA:409L21 siNA (391C) stab10 antisense | GUCAGGAUCCACAGGGCAUATsT | 289 |
| 422 | GAAUAGCCUUCUGAGGAAGGGUA | 250 | | SNCA:442L21 siNA (424C) stab10 antisense | CCCUIUCCUCAGAGGGCAUUTsT | 290 |
| 673 | GUAGCAGGGUUCUUGUGUGCUGU | 251 | | SNCA:693L21 siNA (675C) stab10 antisense | AGCACACAAAGACCCUGCUTsT | 291 |
| 1335 | CCUUCAAUCCUGUCAUUGUUGC | 252 | | SNCA:1355L21 siNA (1337C) stab10 antisense | AAcAUUUGACAGGAUUGAATsT | 292 |
| 673 | GUAGCAGGGUUCUUGUGUGCUGU | 253 | | SNCA:675U21 siNA stab07 sense | B AGcAGGGGucuuuuGuGuGcuTT B | 293 |
| 692 | CUGUGAUUUUGUGGCUUCAUUC | 254 | | SNCA:694U21 siNA stab07 sense | B GuGGAuuuuGuGGcuuCAATT B | 294 |
| 693 | UGUGGAUUUUGUGGCUUCAUUCU | 255 | | SNCA:695U21 siNA stab07 sense | B uGGAUuuuuGuGGcuuCAuTT B | 295 |
| 1335 | CCUUCAAUCCUGUCAUUGUUGC | 256 | | SNCA:1337U21 siNA stab07 sense | B uucaAuccuGucAAuGuuuTT B | 296 |
| 323 | GGCUUUGUCAAAAAGGACCAGUU | 249 | | SNCA:343L21 siNA (325C) stab11 antisense | cuGGuccuuuuuGAcAAAGTsT | 297 |
| 388 | AGAUAGCCUUGUGGAUCCUGACA | 250 | | SNCA:408L21 siNA (390C) stab11 antisense | uCAAGGAuccAcAGGcAuATsT | 298 |
| 389 | GAUAGCCUUGUGGAUCCUGACAA | 251 | | SNCA:409L21 siNA (391C) stab11 antisense | GucAGGAuccAcAGGcAuATsT | 299 |
| 422 | GAAUAGCCUUCUGAGGAAGGGUA | 252 | | SNCA:442L21 siNA (424C) stab11 antisense | cccuuccucAGAAAGGcAuTsT | 300 |
| 673 | GUAGCAGGGUUCUUGUGUGCUGU | 253 | | SNCA:693L21 siNA (675C) stab11 antisense | AGcAcAAAGAcuccuGcuTsT | 301 |
| 692 | CUGUGGAUUUUGUGGCUUCAUUC | 254 | | SNCA:712L21 siNA (694C) stab11 antisense | uuGAAAGccAcAAAuuccAcTsT | 302 |

(400/163)

| | | | | | | |
|------|---------------------------|-----|-------|--|----------------------------|-----|
| 693 | UGUGGAUUUUUGUGGCUUCAAUUCU | 255 | | SNCA:713L21 siNA (695C) stab11 antisense | AuuGAAAGccAcAAAAuuccATsT | 303 |
| 1335 | CCUUCAAUCCUGUCAAUUUUGC | 256 | | SNCA:1355L21 siNA (1337C) stab11 antisense | AAAcAuuGAcAGGAuuGAATsT | 304 |
| 323 | GGCUUUGUCAAAAAGGACCAGUU | 249 | | SNCA:325U21 siNA stab18 sense | B cuuuGucAAAAAGGaccAGTT B | 305 |
| 388 | AGAUAGCCUGUGGAUCCUGACA | 250 | | SNCA:390U21 siNA stab18 sense | B AuAuGccuGuGGAuuccuGATT B | 306 |
| 389 | GAAUAGCCUGUGGAUCCUGACAA | 251 | | SNCA:391U21 siNA stab18 sense | B uAuGccuGuGGAuuccuGATT B | 307 |
| 422 | GAAUAGCCUGUGGAUCCUGACAA | 252 | | SNCA:424U21 siNA stab18 sense | B AAUGccuucucAGGAAGGGTT B | 308 |
| 673 | GUAGCAGGUCUUGUGUGCUGU | 253 | | SNCA:675U21 siNA stab18 sense | B AGcAGGGucuuuuGuGcuTT B | 309 |
| 692 | CUGUGGAUUUUGUGGCUUCAAUUC | 254 | | SNCA:694U21 siNA stab18 sense | B GuGGAuuuuGuGGcuuCAATT B | 310 |
| 693 | UGUGGAUUUUUGUGGCUUCAAUUC | 255 | | SNCA:695U21 siNA stab18 sense | B uGGAuuuuuGuGGcuuCAATT B | 311 |
| 1335 | CCUUCAAUCCUGUCAAUUUUGC | 256 | | SNCA:1337U21 siNA stab18 sense | B uucAAuccuGucAAuGuuuTT B | 312 |
| 323 | GGCUUUGUCAAAAAGGACCAGUU | 249 | | SNCA:343L21 siNA (325C) stab08 antisense | cuGGuccuuuuuGacAAAAGTsT | 313 |
| 388 | AGAUAGCCUGUGGAUCCUGACA | 250 | | SNCA:408L21 siNA (390C) stab08 antisense | ucAGGAuccAcAGGcAuAuTsT | 314 |
| 389 | GAUAUGCCUGUGGAUCCUGACAA | 251 | | SNCA:409L21 siNA (391C) stab08 antisense | GucAGGAuccAcAGGcAuAuTsT | 315 |
| 422 | GAAUAGCCUUCUGAGGAAGGGUA | 252 | | SNCA:442L21 siNA (424C) stab08 antisense | cccuuccucAGAAAGGcAuTsT | 316 |
| 673 | GUAGCAGGUCUUGUGUGCUGU | 253 | | SNCA:693L21 siNA (675C) stab08 antisense | AGcAcAAAAAGAccuGcuTsT | 317 |
| 692 | CUGUGGAUUUUGUGGCUUCAAUUC | 254 | | SNCA:712L21 siNA (694C) stab08 antisense | uuGAAAGccAcAAAAuuccAcTsT | 318 |
| 693 | UGUGGAUUUUUGUGGCUUCAAUUC | 255 | | SNCA:713L21 siNA (695C) stab08 antisense | AuuGAAAGccAcAAAAuuccATsT | 319 |
| 1335 | CCUUCAAUCCUGUCAAUUUUGC | 256 | | SNCA:1355L21 siNA (1337C) stab08 antisense | AAAcAuuGAcAGGAuuGAATsT | 320 |
| 323 | GGCUUUGUCAAAAAGGACCAGUU | 249 | 53969 | SNCA:325U21 siNA stab09 sense | B CUUUUGUCAAAAAGGACCAGTT B | 321 |
| 388 | AGAUAGCCUGUGGAUCCUGACA | 250 | 53970 | SNCA:390U21 siNA stab09 sense | B AUUAUGCCUGUGGAUCCUGATT B | 322 |
| 389 | GAUAUGCCUGUGGAUCCUGACAA | 251 | 53971 | SNCA:391U21 siNA stab09 sense | B UAUGCCUGUGGAUCCUGACTT B | 323 |
| 422 | GAAUAGCCUUCUGAGGAAGGGUA | 252 | 53972 | SNCA:424U21 siNA stab09 sense | B AAUGCCUUCUGAGGAAGGGTT B | 324 |
| 673 | GUAGCAGGUCUUGUGUGCUGU | 253 | 53973 | SNCA:675U21 siNA stab09 sense | B AGCAGGGUCUUUGUGUGCUTT B | 325 |
| 692 | CUGUGGAUUUUGUGGCUUCAAUUC | 254 | 53974 | SNCA:694U21 siNA stab09 sense | B GUGGAUUUUUGUGGCUUCAATT B | 326 |
| 693 | UGUGGAUUUUUGUGGCUUCAAUUC | 255 | 53975 | SNCA:695U21 siNA stab09 sense | B UGGAUUUUUGUGGCUUCAATT B | 327 |
| 1335 | CCUUCAAUCCUGUCAAUUUUGC | 256 | 53976 | SNCA:1337U21 siNA stab09 sense | B UUCAUCCUGUCAAUUUUTT B | 328 |
| 323 | GGCUUUGUCAAAAAGGACCAGUU | 249 | | SNCA:343L21 siNA (325C) stab10 antisense | CUGGUCCUUUUUGACAAAGTsT | 329 |
| 388 | AGAUAGCCUGUGGAUCCUGACA | 250 | | SNCA:408L21 siNA (390C) stab10 antisense | UCAGGAUCCACAGGCAUAUTsT | 330 |

(400/163)

| | | | | | | |
|------|--------------------------|-----|-------|--|----------------------------|-----|
| 389 | GAUAGCCUUGGUAUCCUGACAA | 251 | | SNCA:409L21 siNA (391C) stab10 antisense | GUCAGGAUCCACAGGCAUATsT | 331 |
| 422 | GAAUAGCCUUCUGAGGAAGGGUA | 252 | | SNCA:442L21 siNA (424C) stab10 antisense | CCCUUCCUCAGAAGGCAUUTsT | 332 |
| 673 | GUAGCAGGGUUCUUGUGUGUCUGU | 253 | | SNCA:693L21 siNA (675C) stab10 antisense | AGCACACAAAAGACCCUGCUTsT | 333 |
| 692 | CUGUGGAUUUUGUGGCUUCAAUUC | 254 | | SNCA:712L21 siNA (694C) stab10 antisense | UUUGAAGCCACAAAUAUCCACTsT | 334 |
| 693 | UGUGGAUUUUGUGGCUUCAAUUC | 255 | | SNCA:713L21 siNA (695C) stab10 antisense | AUUGAAGCCACAAAUAUCCATsT | 335 |
| 1335 | CCUCAAUCCUGUCAAUGUUUGC | 256 | | SNCA:1355L21 siNA (1337C) stab10 antisense | AAACAUUGACAGGGAUUGAATsT | 336 |
| 323 | GGCUUUGUCAAAAAGGACCAGUU | 249 | 53977 | SNCA:343L21 siNA (325C) stab19 antisense | cuGGuccuuuuuGACAAAAGTT B | 337 |
| 388 | AGAUAGCCUUGUGGAUCCUGACA | 250 | 53978 | SNCA:408L21 siNA (390C) stab19 antisense | ucAGGAuccAcAGGcAUuTT B | 338 |
| 389 | GAUAGCCUUGUGGAUCCUGACAA | 251 | 53979 | SNCA:409L21 siNA (391C) stab19 antisense | GucAGGAuccAcAGGcAUuTT B | 339 |
| 422 | GAAUAGCCUUCUGAGGAAGGGUA | 252 | 53980 | SNCA:442L21 siNA (424C) stab19 antisense | ccuuuccuAGAAAGGcAUuTT B | 340 |
| 673 | GUAGCAGGGUUCUUGUGUGUCUGU | 253 | 53981 | SNCA:693L21 siNA (675C) stab19 antisense | AGcAcAAAAGAcccuGcuTT B | 341 |
| 692 | CUGUGGAUUUUGUGGCUUCAAUUC | 254 | 53982 | SNCA:712L21 siNA (694C) stab19 antisense | uuGAAAGccAcAAAuuccAcTT B | 342 |
| 693 | UGUGGAUUUUGUGGCUUCAAUUC | 255 | 53983 | SNCA:713L21 siNA (695C) stab19 antisense | AuuGAAAGccAcAAAuuccAcTT B | 343 |
| 1335 | CCUCAAUCCUGUCAAUGUUUGC | 256 | 53984 | SNCA:1355L21 siNA (1337C) stab19 antisense | AAAcAuuGAcAGGGAuuGAAAT B | 344 |
| 323 | GGCUUUGUCAAAAAGGACCAGUU | 249 | 53985 | SNCA:343L21 siNA (325C) stab22 antisense | CUGGUCCUUUUUGACAAAAGTT B | 345 |
| 388 | AGAUAGCCUUGUGGAUCCUGACA | 250 | 53986 | SNCA:408L21 siNA (390C) stab22 antisense | UCAGGAUCCACACAGGCAUuTT B | 346 |
| 389 | GAUAGCCUUGUGGAUCCUGACAA | 251 | 53987 | SNCA:409L21 siNA (391C) stab22 antisense | GUCAGGAUCCACAGGCAUuTT B | 347 |
| 422 | GAAUAGCCUUCUGAGGAAGGGUA | 252 | 53988 | SNCA:442L21 siNA (424C) stab22 antisense | CCCUUCCUCAGAAGGCAUuTT B | 348 |
| 673 | GUAGCAGGGUUCUUGUGUGUCUGU | 253 | 53989 | SNCA:693L21 siNA (675C) stab22 antisense | AGCACACAAAAGACCCUGCUTT B | 349 |
| 692 | CUGUGGAUUUUGUGGCUUCAAUUC | 254 | 53990 | SNCA:712L21 siNA (694C) stab22 antisense | UUUGAAGCCACAAAUAUCCACTT B | 350 |
| 693 | UGUGGAUUUUGUGGCUUCAAUUC | 255 | 53991 | SNCA:713L21 siNA (695C) stab22 antisense | AUUGAAGCCACACAAAUAUCCATT B | 351 |

(400/163)

| | | | | | | |
|------|------------------------|-----|-------|---|---------------------------|-----|
| 1335 | CCUUCAAUCCUGUCAAGUUUGC | 256 | 53992 | SNCA:1355L21 siNA (1337C) stab22 antisense | AAACAUUGACACGGAUUUGAATT B | 352 |
|------|------------------------|-----|-------|---|---------------------------|-----|

| |
|------------------------------|
| Uppercase = ribonucleotide |
| u,c = 2'-deoxy-2'-fluoro U,C |
| T = thymidine |
| B = inverted deoxy abasic |
| s = phosphorothioate linkage |
| A = deoxy Adenosine |
| G = deoxy Guanosine |
| G = 2'-O-methyl Guanosine |
| A = 2'-O-methyl Adenosine |

Table IV

Non-limiting examples of Stabilization Chemistries for chemically modified siNA constructs

| Chemistry | pyrimidine | Purine | cap | p=S | Strand |
|-----------|-------------|--------------|----------------|----------------------------|------------|
| "Stab 00" | Ribo | Ribo | TT at 3'-ends | | S/AS |
| "Stab 1" | Ribo | Ribo | - | 5 at 5'-end 1 at 3'-end | S/AS |
| "Stab 2" | Ribo | Ribo | - | All linkages | Usually AS |
| "Stab 3" | 2'-fluoro | Ribo | - | 4 at 5'-end 4 at 3'-end | Usually S |
| "Stab 4" | 2'-fluoro | Ribo | 5' and 3'-ends | - | Usually S |
| "Stab 5" | 2'-fluoro | Ribo | - | 1 at 3'-end | Usually AS |
| "Stab 6" | 2'-O-Methyl | Ribo | 5' and 3'-ends | - | Usually S |
| "Stab 7" | 2'-fluoro | 2'-deoxy | 5' and 3'-ends | - | Usually S |
| "Stab 8" | 2'-fluoro | 2'-O-Methyl | - | 1 at 3'-end | Usually AS |
| "Stab 9" | Ribo | Ribo | 5' and 3'-ends | - | Usually S |
| "Stab 10" | Ribo | Ribo | - | 1 at 3'-end | Usually AS |
| "Stab 11" | 2'-fluoro | 2'-deoxy | - | 1 at 3'-end | Usually AS |
| "Stab 12" | 2'-fluoro | LNA | 5' and 3'-ends | | Usually S |
| "Stab 13" | 2'-fluoro | LNA | | 1 at 3'-end | Usually AS |
| "Stab 14" | 2'-fluoro | 2'-deoxy | | 2 at 5'-end 1 at 3'-end | Usually AS |
| "Stab 15" | 2'-deoxy | 2'-deoxy | | 2 at 5'-end 1 at 3'-end | Usually AS |
| "Stab 16" | Ribo | 2'-O-Methyl | 5' and 3'-ends | | Usually S |
| "Stab 17" | 2'-O-Methyl | 2'-O-Methyl | 5' and 3'-ends | | Usually S |
| "Stab 18" | 2'-fluoro | 2'-O-Methyl | 5' and 3'-ends | 1 at 3'-end | Usually S |
| "Stab 19" | 2'-fluoro | 2'-O-Methyl | 3'-end | | Usually AS |
| "Stab 20" | 2'-fluoro | 2'-deoxy | 3'-end | | Usually AS |
| "Stab 21" | 2'-fluoro | Ribo | 3'-end | | Usually AS |
| "Stab 22" | Ribo | Ribo | 3'-end - | | Usually AS |
| "Stab 23" | 2'-fluoro* | 2'-deoxy* | 5' and 3'-ends | | Usually S |
| "Stab 24" | 2'-fluoro* | 2'-O-Methyl* | - | 1 at 3'-end | Usually AS |
| "Stab 25" | 2'-fluoro* | 2'-O- | - | 1 at 3'-end | Usually AS |

| | | | | | |
|--|--|---------|--|--|--|
| | | Methyl* | | | |
|--|--|---------|--|--|--|

CAP = any terminal cap, see for example **Figure 10**.

All Stab 1-25 chemistries can comprise 3'-terminal thymidine (TT) residues

All Stab 1-25 chemistries typically comprise about 21 nucleotides, but can vary as described herein

S = sense strand

AS = antisense strand

*Stab 23 has single ribonucleotide adjacent to 3'-CAP

*Stab 24 has single ribonucleotide at 5'-terminus

*Stab 25 has three ribonucleotides at 5'-terminus

Table V

A. 2.5 μ mol Synthesis Cycle ABI 394 Instrument

| Reagent | Equivalents | Amount | Wait Time* DNA | Wait Time* 2'-O-methyl | Wait Time*RNA |
|--------------------|-------------|-------------|----------------|------------------------|---------------|
| Phosphoramidites | 6.5 | 163 μ L | 45 sec | 2.5 min | 7.5 min |
| S-Ethyl Tetrazole | 23.8 | 238 μ L | 45 sec | 2.5 min | 7.5 min |
| Acetic Anhydride | 100 | 233 μ L | 5 sec | 5 sec | 5 sec |
| N-Methyl Imidazole | 186 | 233 μ L | 5 sec | 5 sec | 5 sec |
| TCA | 176 | 2.3 mL | 21 sec | 21 sec | 21 sec |
| Iodine | 11.2 | 1.7 mL | 45 sec | 45 sec | 45 sec |
| Beaucage | 12.9 | 645 μ L | 100 sec | 300 sec | 300 sec |
| Acetonitrile | NA | 6.67 mL | NA | NA | NA |

B. 0.2 μ mol Synthesis Cycle ABI 394 Instrument

| Reagent | Equivalents | Amount | Wait Time* DNA | Wait Time* 2'-O-methyl | Wait Time*RNA |
|--------------------|-------------|-------------|----------------|------------------------|---------------|
| Phosphoramidites | 15 | 31 μ L | 45 sec | 233 sec | 465 sec |
| S-Ethyl Tetrazole | 38.7 | 31 μ L | 45 sec | 233 min | 465 sec |
| Acetic Anhydride | 655 | 124 μ L | 5 sec | 5 sec | 5 sec |
| N-Methyl Imidazole | 1245 | 124 μ L | 5 sec | 5 sec | 5 sec |
| TCA | 700 | 732 μ L | 10 sec | 10 sec | 10 sec |
| Iodine | 20.6 | 244 μ L | 15 sec | 15 sec | 15 sec |
| Beaucage | 7.7 | 232 μ L | 100 sec | 300 sec | 300 sec |
| Acetonitrile | NA | 2.64 mL | NA | NA | NA |

C. 0.2 μ mol Synthesis Cycle 96 well Instrument

| Reagent | Equivalents:DNA/ 2'-O-methyl/Ribo | Amount: DNA/2'-O- methyl/Ribo | Wait Time* DNA | Wait Time* 2'-O- methyl | Wait Time* Ribo |
|--------------------|--------------------------------------|----------------------------------|----------------|----------------------------|-----------------|
| Phosphoramidites | 22/33/66 | 40/60/120 μ L | 60 sec | 180 sec | 360sec |
| S-Ethyl Tetrazole | 70/105/210 | 40/60/120 μ L | 60 sec | 180 min | 360 sec |
| Acetic Anhydride | 265/265/265 | 50/50/50 μ L | 10 sec | 10 sec | 10 sec |
| N-Methyl Imidazole | 502/502/502 | 50/50/50 μ L | 10 sec | 10 sec | 10 sec |
| TCA | 238/475/475 | 250/500/500 μ L | 15 sec | 15 sec | 15 sec |
| Iodine | 6.8/6.8/6.8 | 80/80/80 μ L | 30 sec | 30 sec | 30 sec |
| Beaucage | 34/51/51 | 80/120/120 | 100 sec | 200 sec | 200 sec |
| Acetonitrile | NA | 1150/1150/1150 μ L | NA | NA | NA |

5 • Wait time does not include contact time during delivery.

• Tandem synthesis utilizes double coupling of linker molecule

CLAIMS

What we claim is:

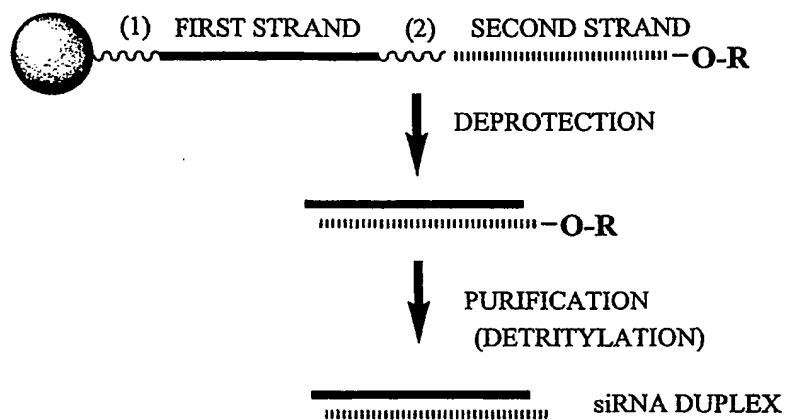
1. A chemically synthesized double stranded short interfering nucleic acid (siNA) molecule that directs cleavage of a alpha-synuclein (SNCA) RNA via RNA interference (RNAi), wherein:
 - a. each strand of said siNA molecule is about 19 to about 23 nucleotides in length; and
 - b. one strand of said siNA molecule comprises nucleotide sequence having sufficient complementarity to said SNCA RNA for the siNA molecule to direct cleavage of the SNCA RNA via RNA interference.
2. The siNA molecule of claim 1, wherein said siNA molecule comprises no ribonucleotides.
3. The siNA molecule of claim 1, wherein said siNA molecule comprises one or more ribonucleotides.
4. The siNA molecule of claim 1, wherein one strand of said double-stranded siNA molecule comprises a nucleotide sequence that is complementary to a nucleotide sequence of a SNCA gene or a portion thereof, and wherein a second strand of said double-stranded siNA molecule comprises a nucleotide sequence substantially similar to the nucleotide sequence or a portion thereof of said SNCA RNA.
5. The siNA molecule of claim 4, wherein each strand of the siNA molecule comprises about 19 to about 23 nucleotides, and wherein each strand comprises at least about 19 nucleotides that are complementary to the nucleotides of the other strand.
6. The siNA molecule of claim 1, wherein said siNA molecule comprises an antisense region comprising a nucleotide sequence that is complementary to a nucleotide sequence of a SNCA gene or a portion thereof, and wherein said siNA further comprises a sense region, wherein said sense region comprises a nucleotide sequence substantially similar to the nucleotide sequence of said SNCA gene or a portion thereof.
7. The siNA molecule of claim 6, wherein said antisense region and said sense region comprise about 19 to about 23 nucleotides, and wherein said antisense region

comprises at least about 19 nucleotides that are complementary to nucleotides of the sense region.

8. The siNA molecule of claim 1, wherein said siNA molecule comprises a sense region and an antisense region, and wherein said antisense region comprises a nucleotide sequence that is complementary to a nucleotide sequence of RNA encoded by a SNCA gene, or a portion thereof, and said sense region comprises a nucleotide sequence that is complementary to said antisense region.
9. The siNA molecule of claim 6, wherein said siNA molecule is assembled from two separate oligonucleotide fragments wherein one fragment comprises the sense region and a second fragment comprises the antisense region of said siNA molecule.
10. The siNA molecule of claim 6, wherein said sense region is connected to the antisense region via a linker molecule.
11. The siNA molecule of claim 10, wherein said linker molecule is a polynucleotide linker.
12. The siNA molecule of claim 10, wherein said linker molecule is a non-nucleotide linker.
13. The siNA molecule of claim 6, wherein pyrimidine nucleotides in the sense region are 2'-O-methyl pyrimidine nucleotides.
14. The siNA molecule of claim 6, wherein purine nucleotides in the sense region are 2'-deoxy purine nucleotides.
15. The siNA molecule of claim 6, wherein pyrimidine nucleotides present in the sense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides.
16. The siNA molecule of claim 9, wherein the fragment comprising said sense region includes a terminal cap moiety at a 5'-end, a 3'-end, or both of the 5' and 3' ends of the fragment comprising said sense region.
17. The siNA molecule of claim 16, wherein said terminal cap moiety is an inverted deoxy abasic moiety.
18. The siNA molecule of claim 6, wherein pyrimidine nucleotides of said antisense region are 2'-deoxy-2'-fluoro pyrimidine nucleotides.

19. The siNA molecule of claim 6, wherein purine nucleotides of said antisense region are 2'-O-methyl purine nucleotides.
20. The siNA molecule of claim 6, wherein purine nucleotides present in said antisense region comprise 2'-deoxy- purine nucleotides.
21. The siNA molecule of claim 18, wherein said antisense region comprises a phosphorothioate internucleotide linkage at the 3' end of said antisense region.
22. The siNA molecule of claim 6, wherein said antisense region comprises a glyceryl modification at a 3' end of said antisense region.
23. The siNA molecule of claim 9, wherein each of the two fragments of said siNA molecule comprise about 21 nucleotides.
24. The siNA molecule of claim 23, wherein about 19 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule and wherein at least two 3' terminal nucleotides of each fragment of the siNA molecule are not base-paired to the nucleotides of the other fragment of the siNA molecule.
25. The siNA molecule of claim 24, wherein each of the two 3' terminal nucleotides of each fragment of the siNA molecule are 2'-deoxy-pyrimidines.
26. The siNA molecule of claim 25, wherein said 2'-deoxy-pyrimidine is 2'-deoxy-thymidine.
27. The siNA molecule of claim 23, wherein all of the about 21 nucleotides of each fragment of the siNA molecule are base-paired to the complementary nucleotides of the other fragment of the siNA molecule.
28. The siNA molecule of claim 23, wherein about 19 nucleotides of the antisense region are base-paired to the nucleotide sequence of the RNA encoded by a SNCA gene or a portion thereof.
29. The siNA molecule of claim 23, wherein about 21 nucleotides of the antisense region are base-paired to the nucleotide sequence of the RNA encoded by a SNCA gene or a portion thereof.
30. The siNA molecule of claim 9, wherein a 5'-end of the fragment comprising said antisense region optionally includes a phosphate group.

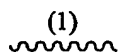
31. A composition comprising the siNA molecule of claim 1 in an pharmaceutically acceptable carrier or diluent.
32. A siNA according to claim 1 wherein the SNCA RNA comprises Genebank Accession No. NM_000345.
33. A siNA according to claim 1 wherein said siNA comprises any of SEQ ID NOs. 1-86, 173-210, 249-252, 253-256, 261-264, 269-272, 277-280, 285-288, 293-296, 305-312, 321-328, 353, 355, 357, 359, 360, 362, 364, 366, 368, 369, 87-172, 211-248, 257-260, 265-268, 273-276, 281-284, 289-292, 297-304, 313-320, 329-352, 354, 356, 358, 361, 363, 365, 367, or 370.
34. A composition comprising the siNA of claim 32 together with a pharmaceutically acceptable carrier or diluent.
35. A composition comprising the siNA of claim 33 together with a pharmaceutically acceptable carrier or diluent.

Figure 1

= SOLID SUPPORT

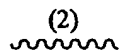
R = TERMINAL PROTECTING GROUP

FOR EXAMPLE:
DIMETHOXYTRITYL (DMT)



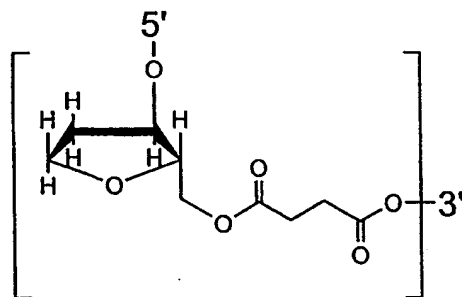
(1) = CLEAVABLE LINKER

(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR
INVERTED DEOXYABASIC SUCCINATE)

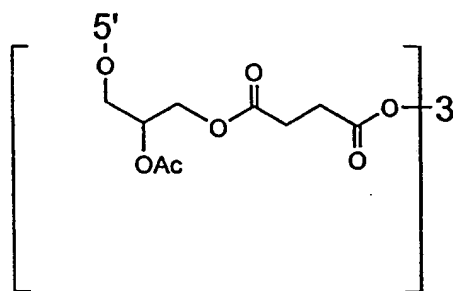


(2) = CLEAVABLE LINKER

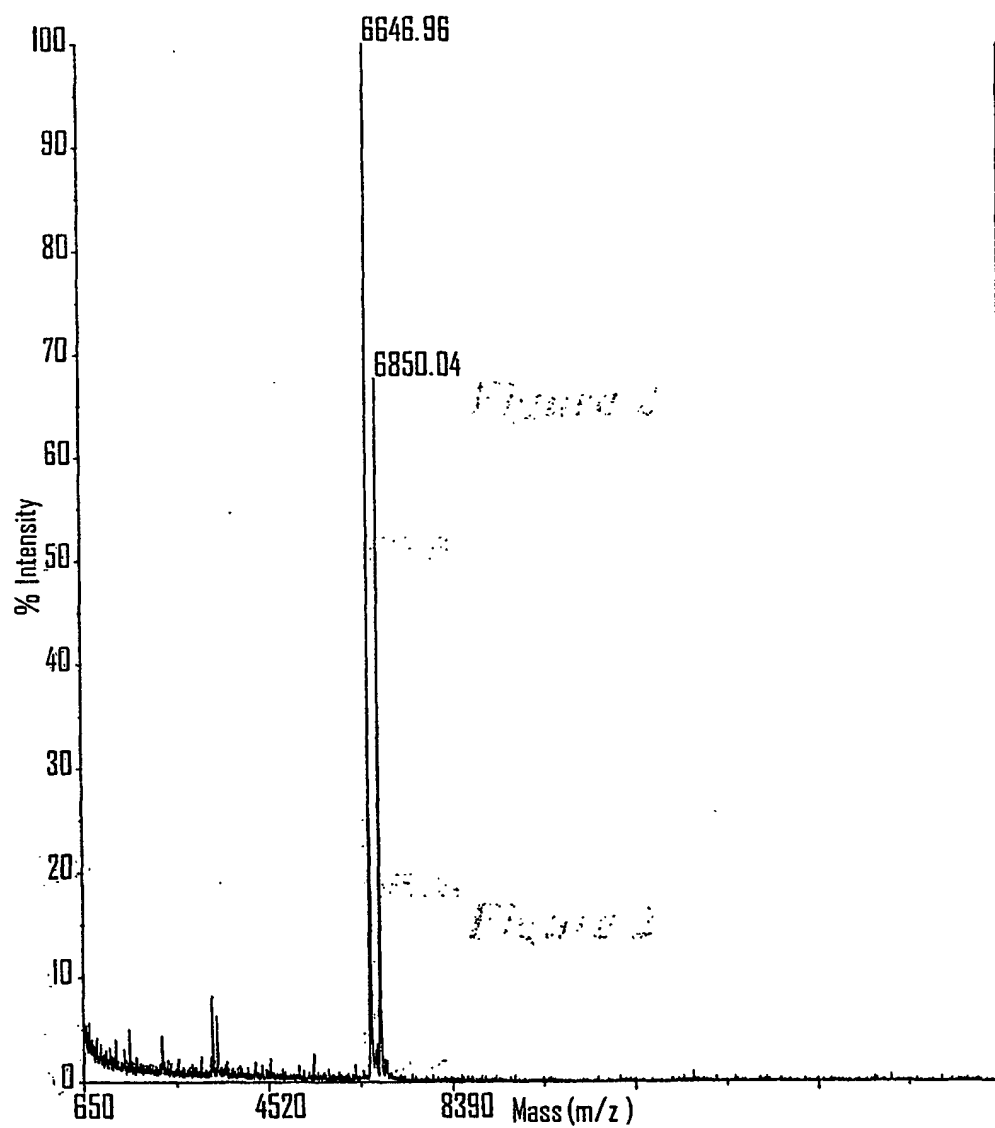
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR
INVERTED DEOXYABASIC SUCCINATE)



INVERTED DEOXYABASIC SUCCINATE
LINKAGE

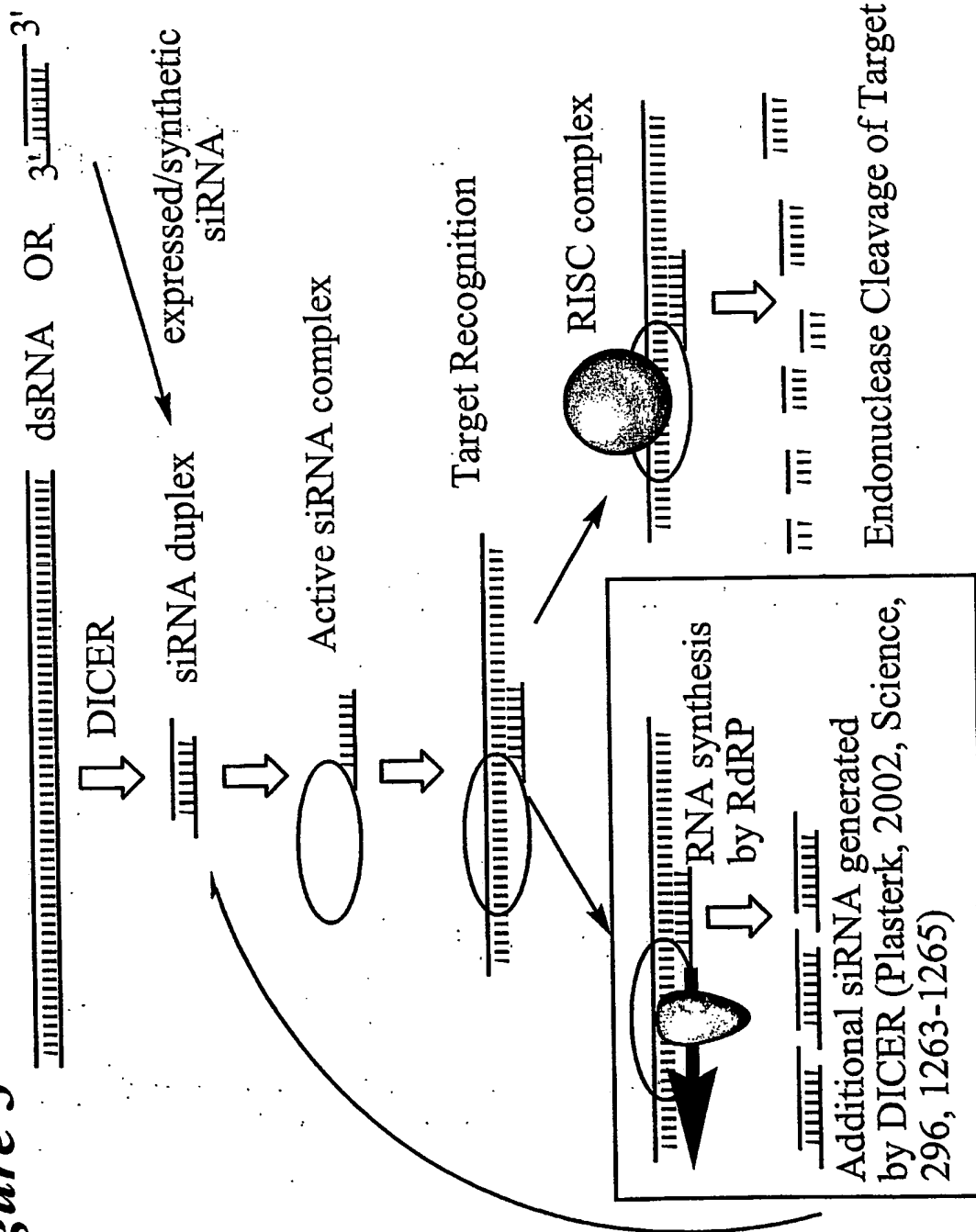


GLYCERYL SUCCINATE LINKAGE

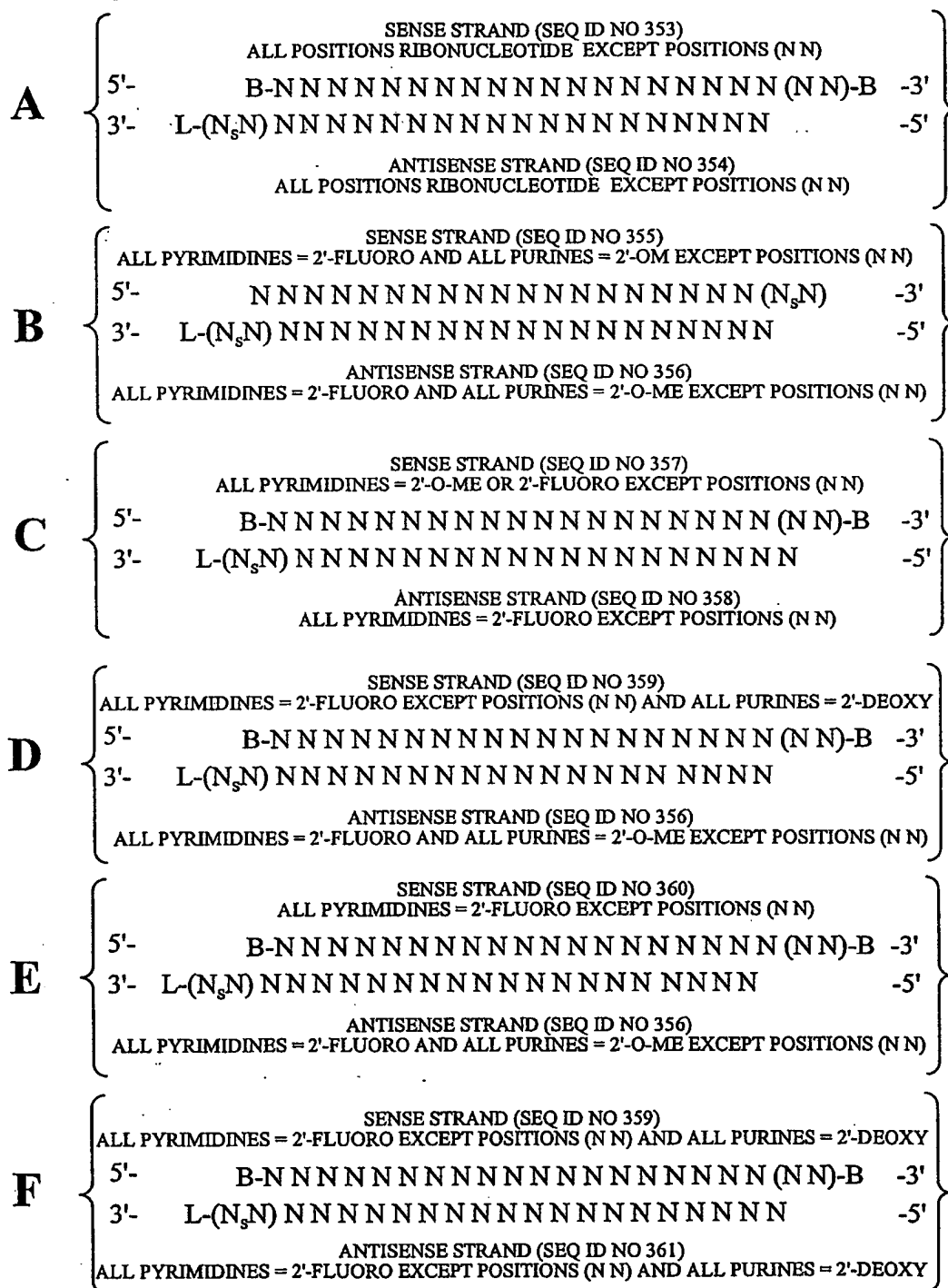
Figure 2

3/24

Figure 3

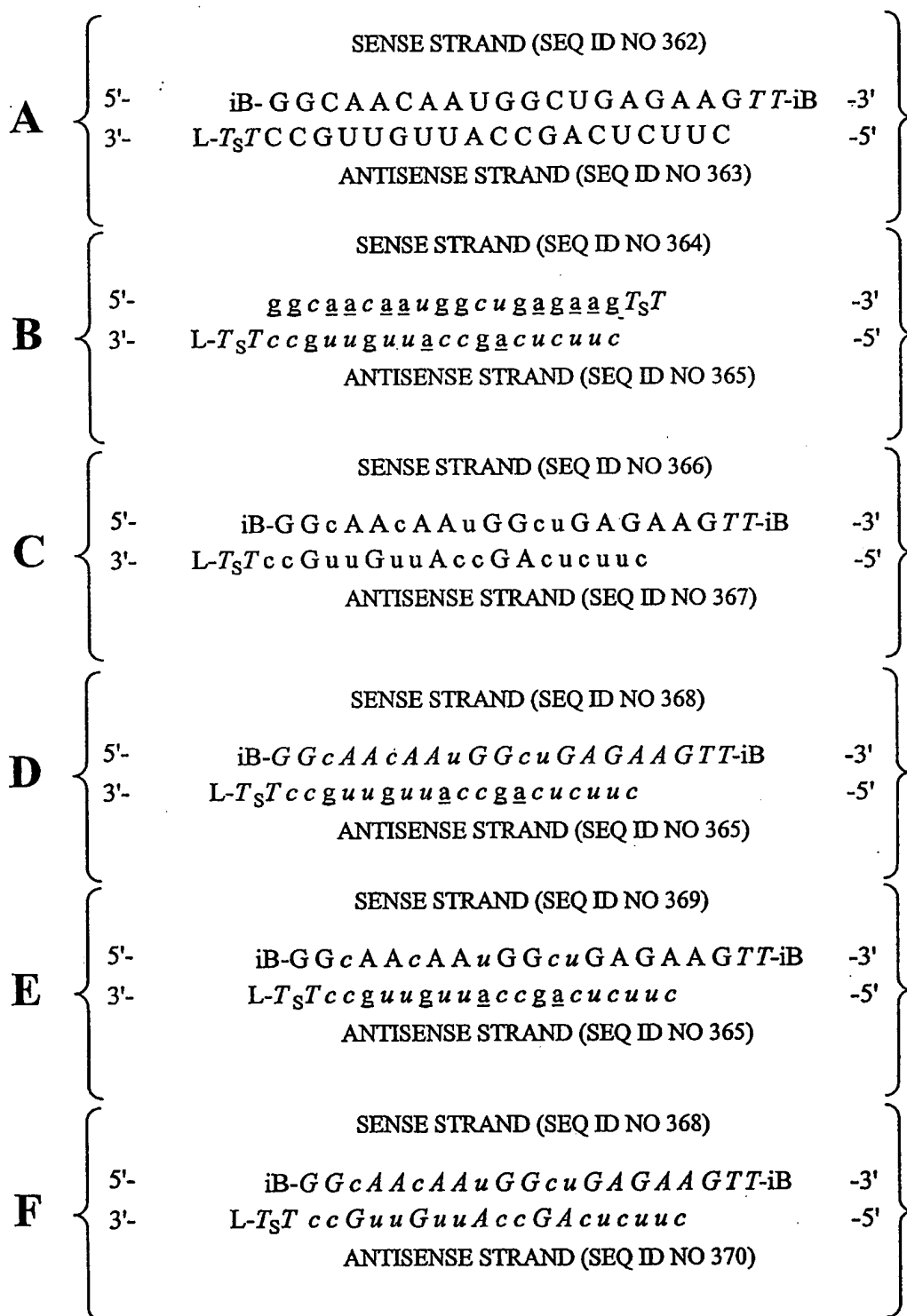


4/24

Figure 4

POSITIONS (NN) CAN COMPRISE ANY NUCLEOTIDE, SUCH AS DEOXYNUCLEOTIDES (eg. THYMIDINE) OR UNIVERSAL BASES
 B = ABASIC, INVERTED ABASIC, INVERTED NUCLEOTIDE OR OTHER TERMINAL CAP THAT IS OPTIONALLY PRESENT
 L = GLYCERYL or B THAT IS OPTIONALLY PRESENT
 S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE that is optionally absent

5/24

Figure 5

lower case = 2'-O-Methyl or 2'-deoxy-2'-fluoro

italic lower case = 2'-deoxy-2'-fluorounderline = 2'-O-methyl*ITALIC UPPER CASE* = DEOXY

iB = INVERTED DEOXYABASIC

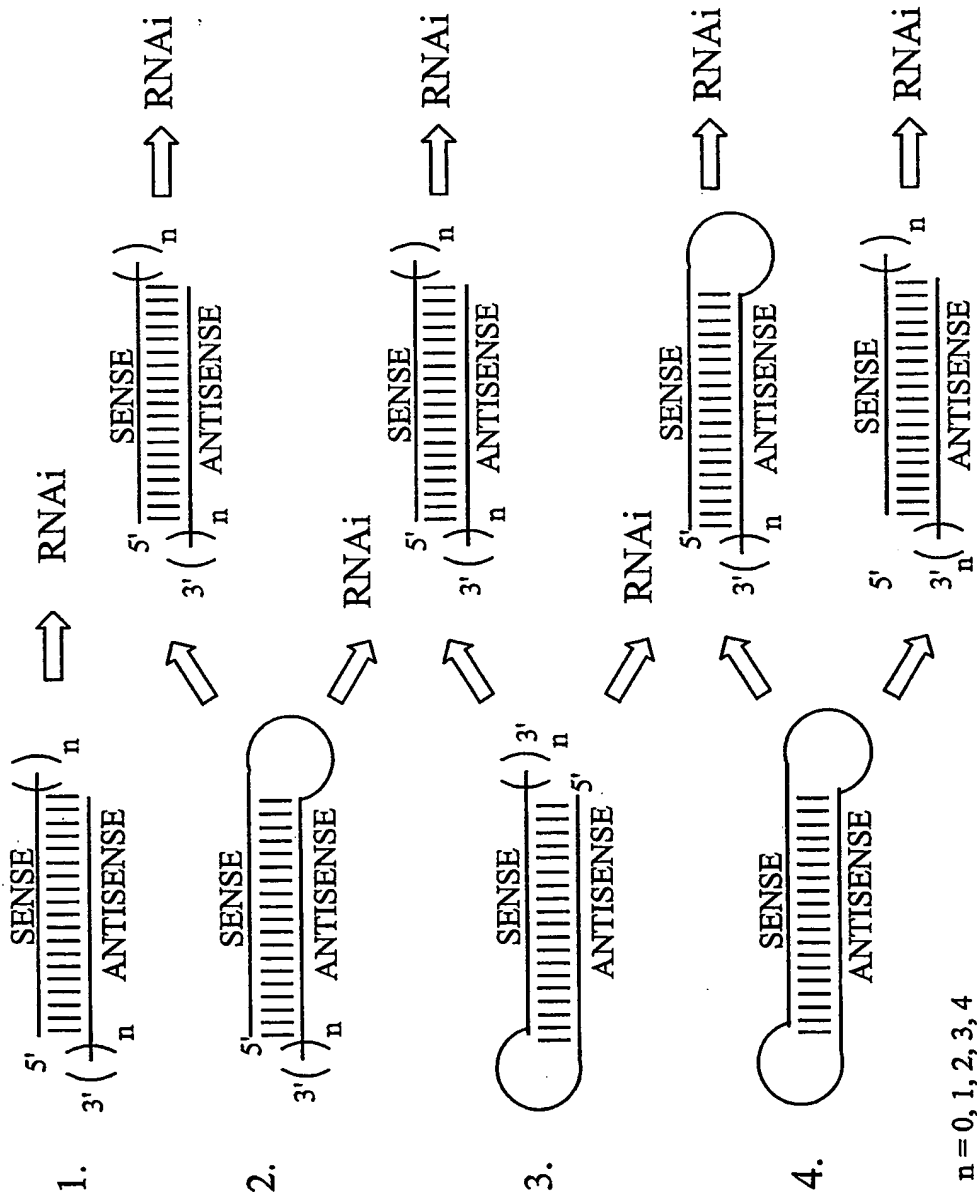
L = GLYCERYL MOIETY or iB OPTIONALLY PRESENT

S = PHOSPHOROTHIOATE OR

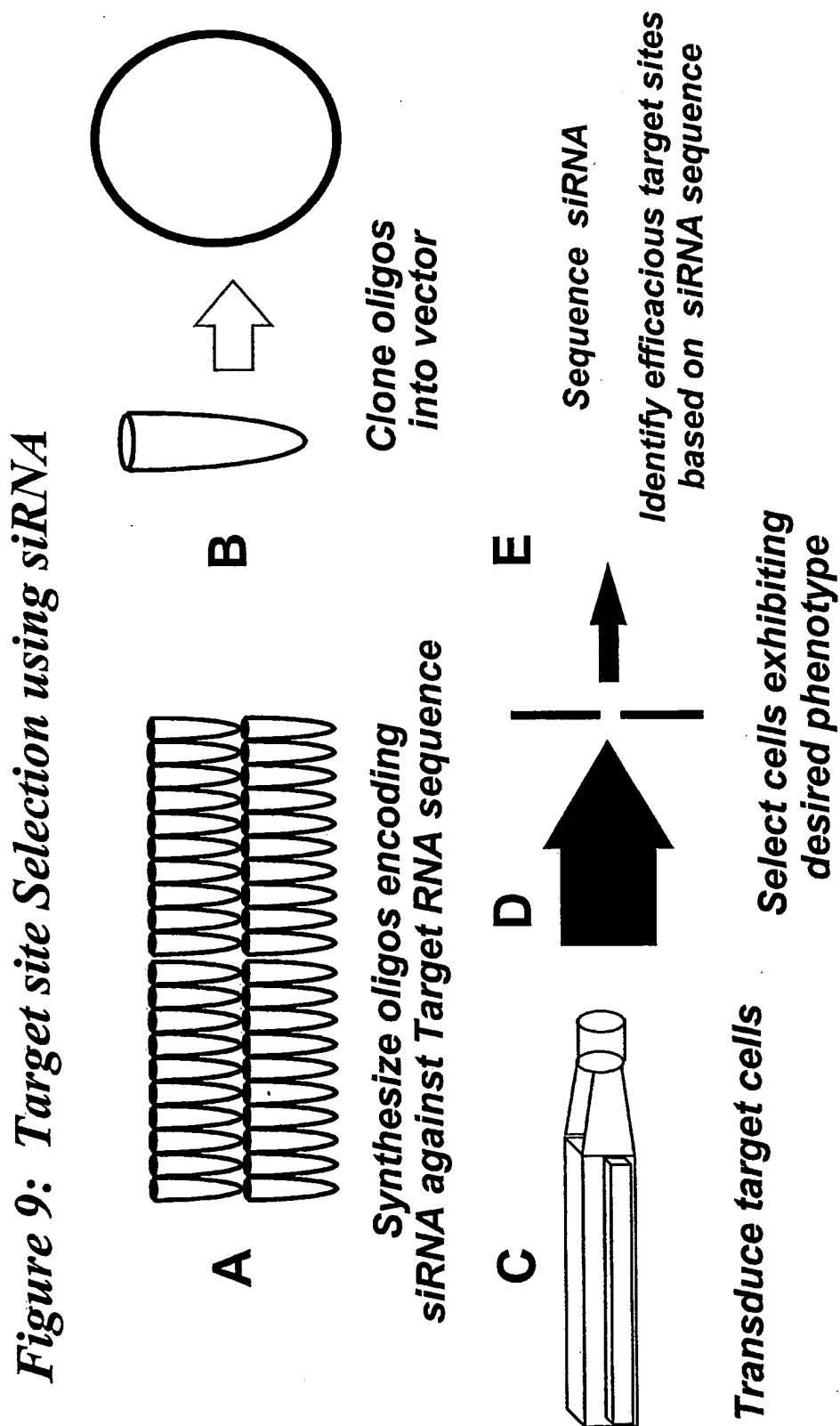
PHOSPHORODITHIOATE OPTIONALLY PRESENT

6/24

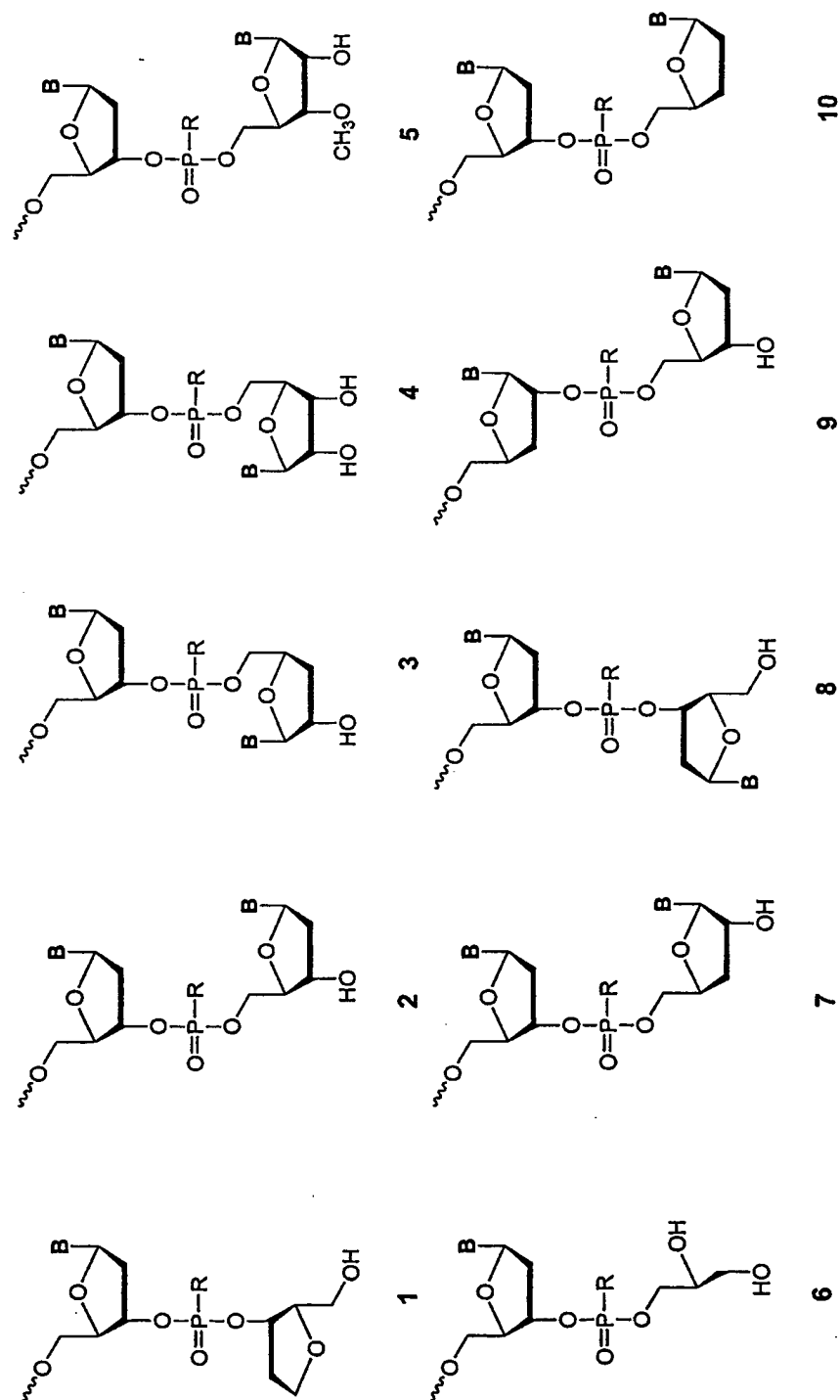
Figure 6



9/24



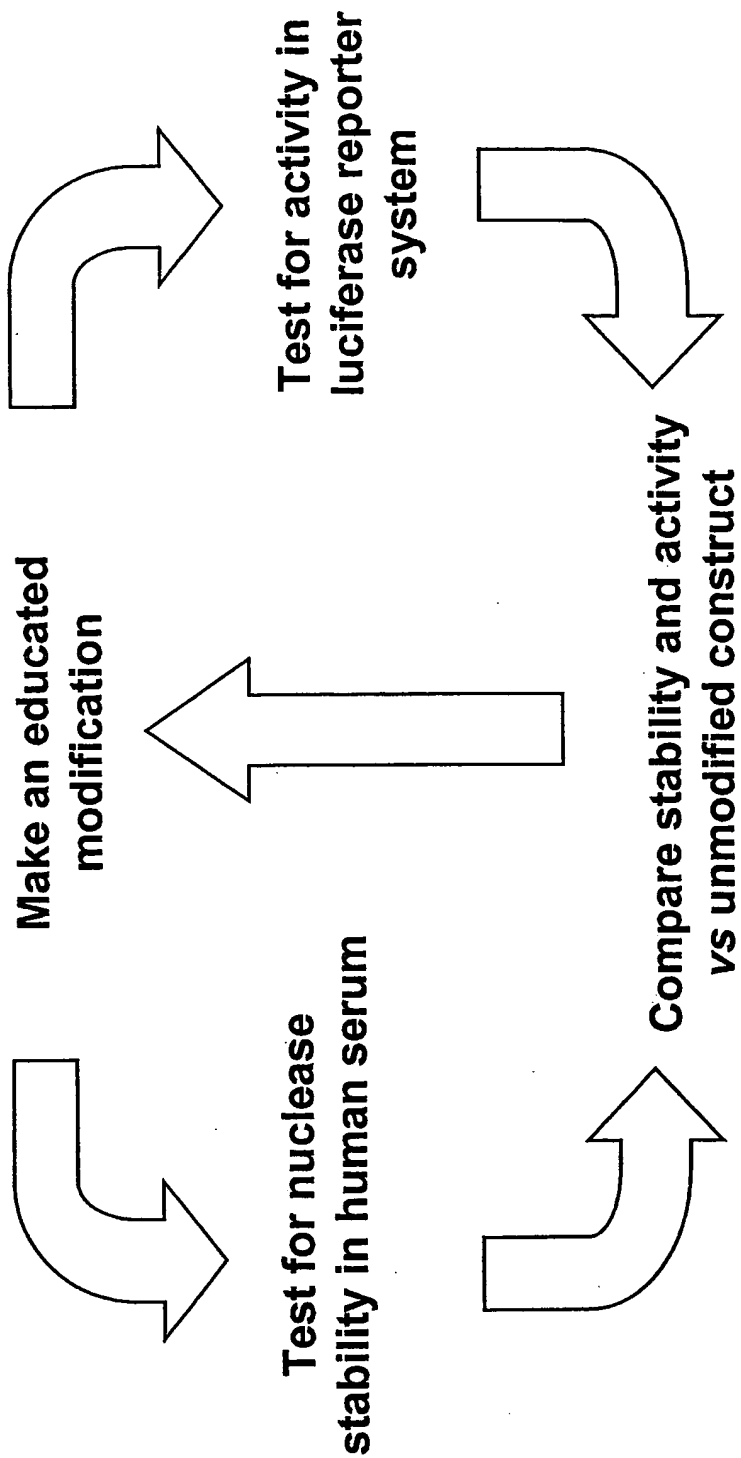
10/24

Figure 10

R = O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, or aralkyl
B = Independently any nucleotide base, either naturally occurring or chemically modified, or optionally H (abasic).

11/24

Figure 11: Modification Strategy



12/24

Figure 12: Phosphorylated siNA constructs

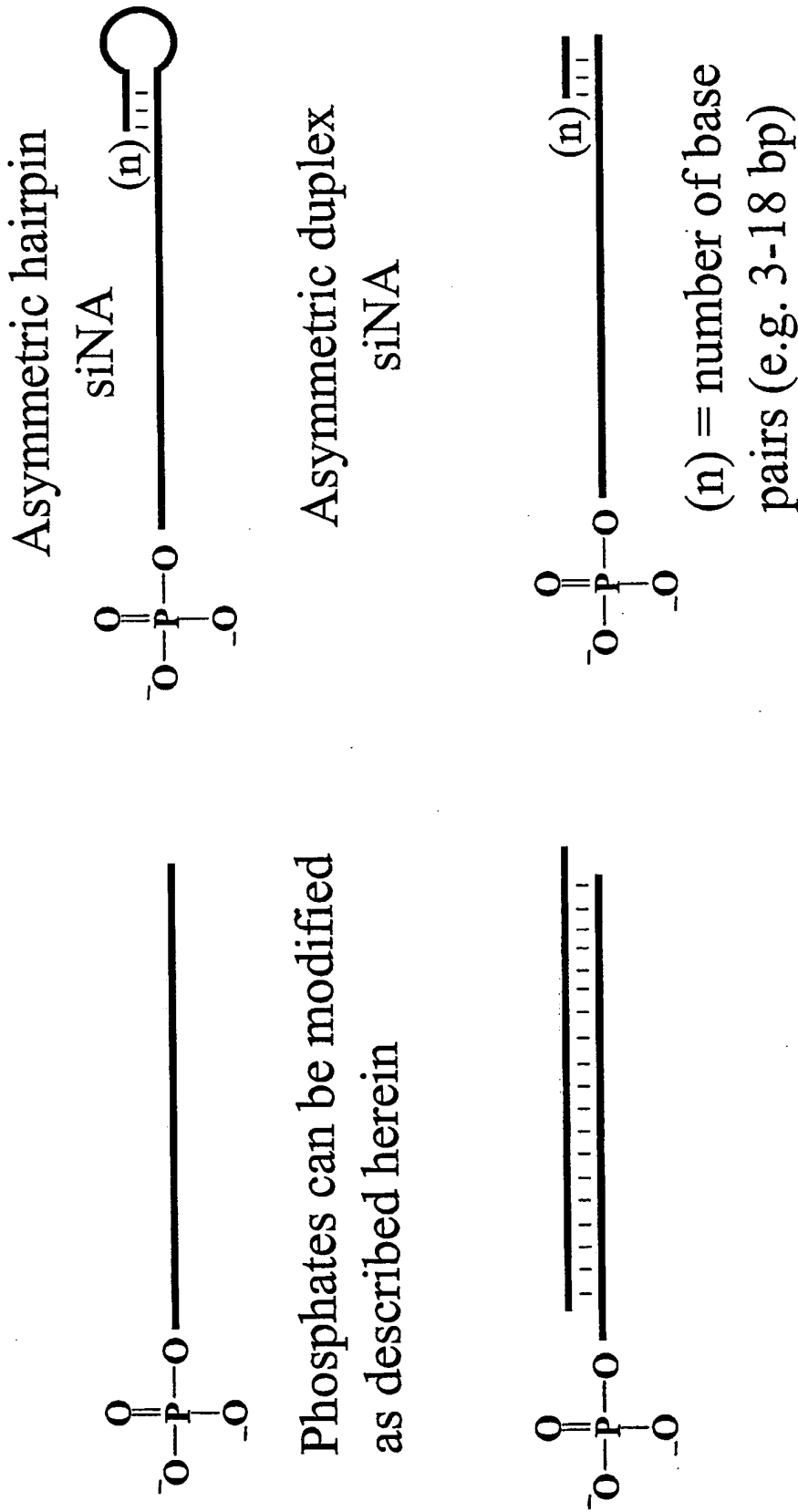
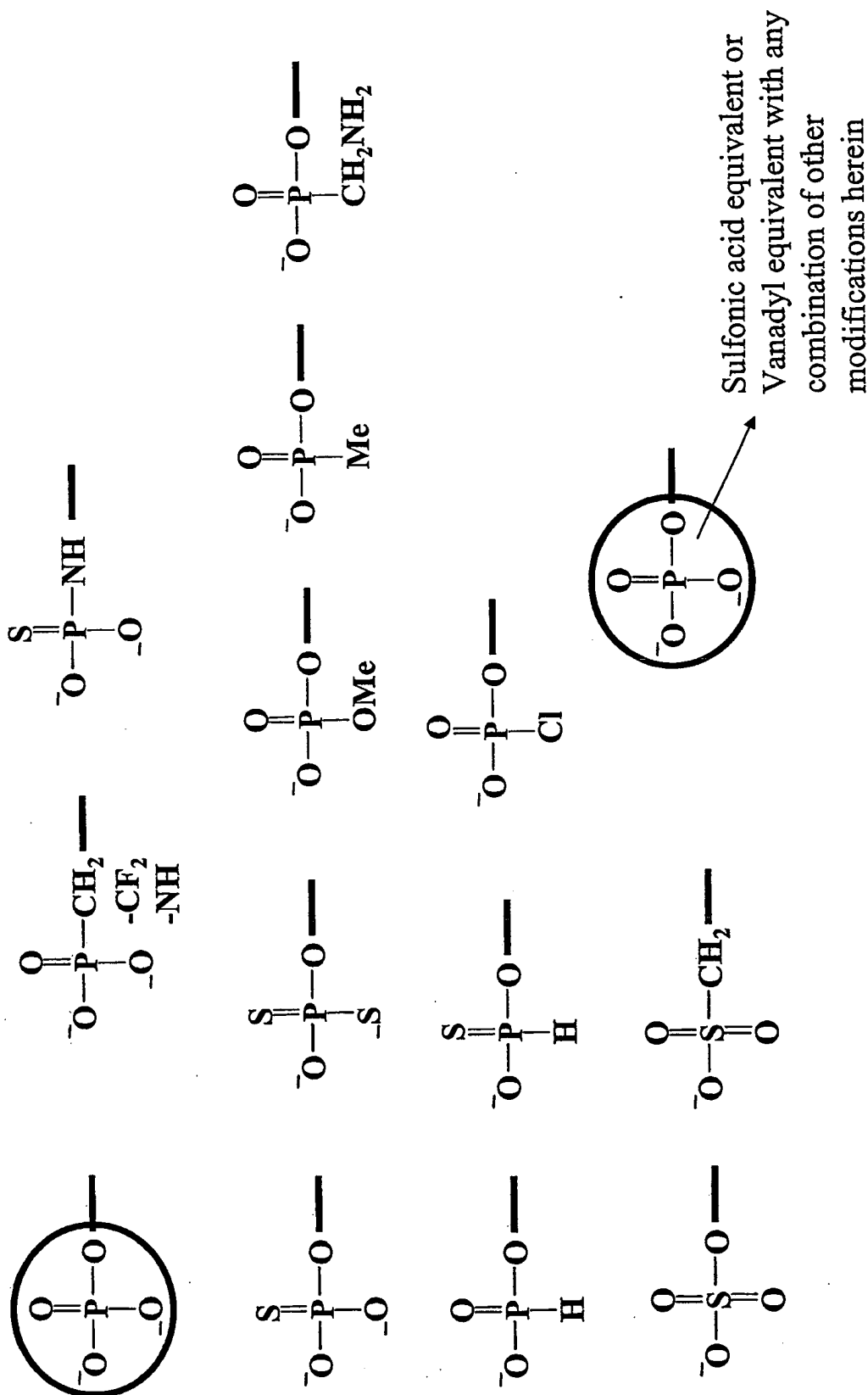


Figure 13: 5'-phosphate modifications



14/24

Figure 14A: Duplex forming oligonucleotide constructs that utilize palindrome or repeat sequences

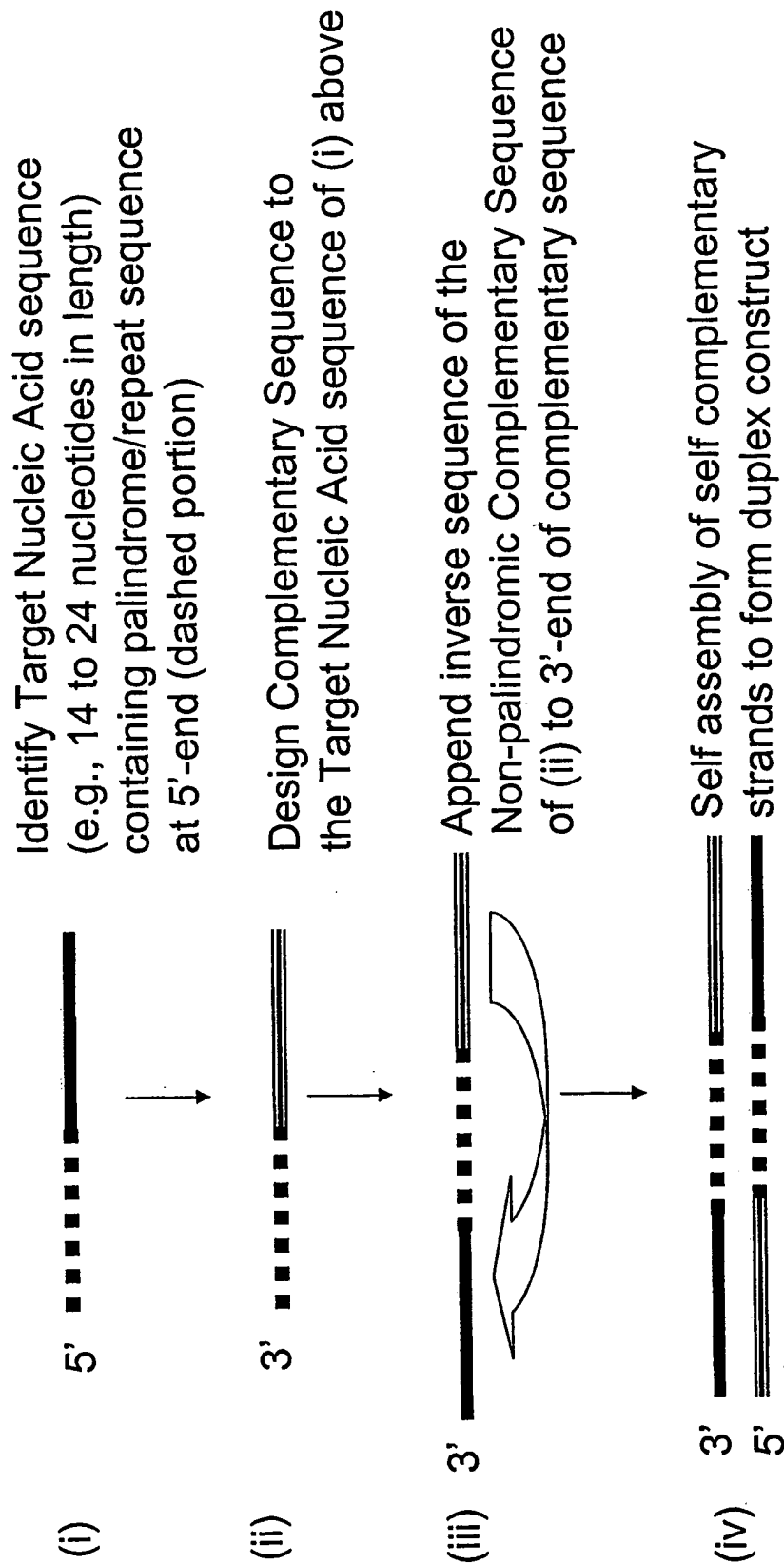
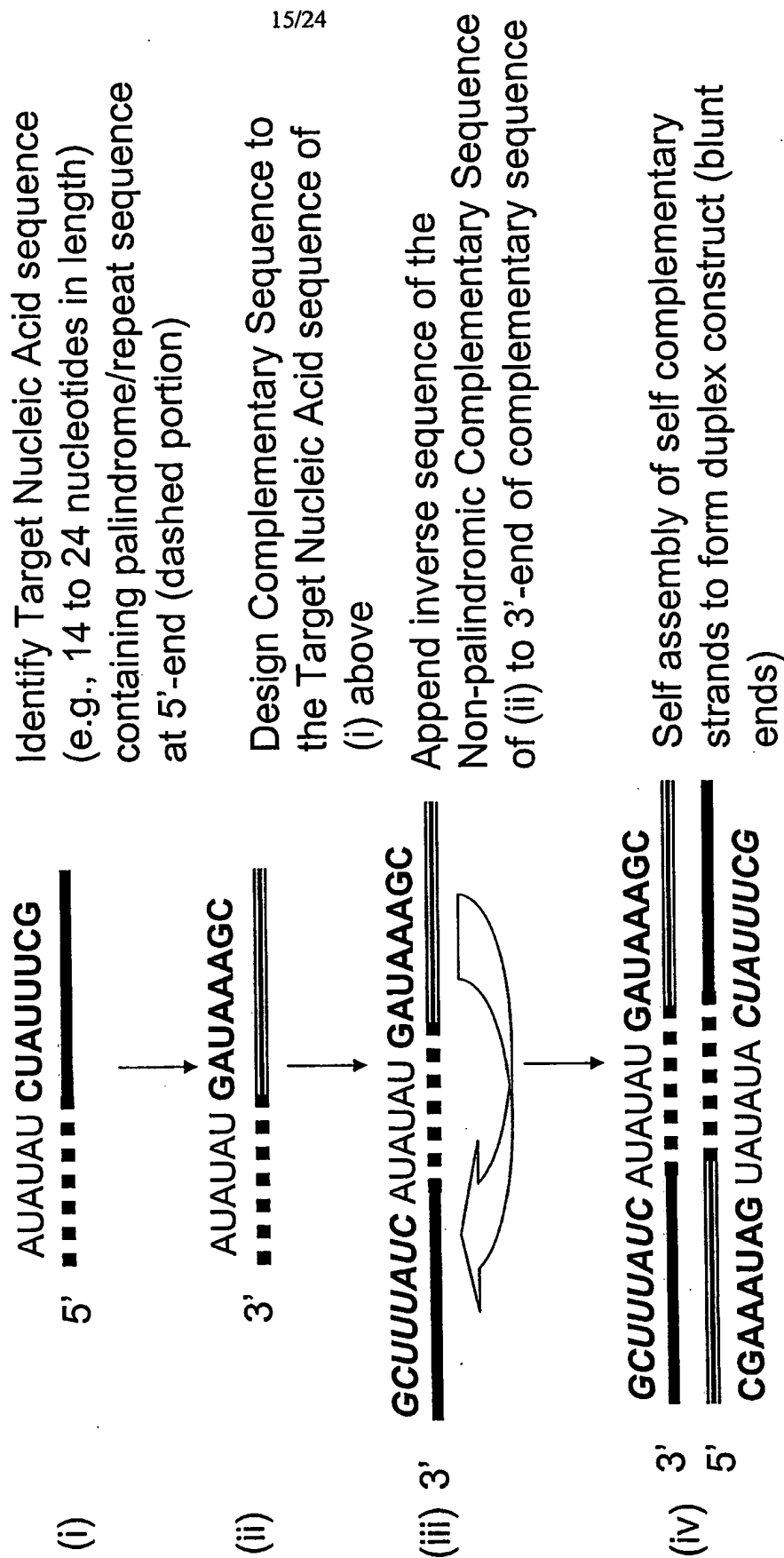


Figure 14B: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence



16/24

Figure 14C: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence, self assembly

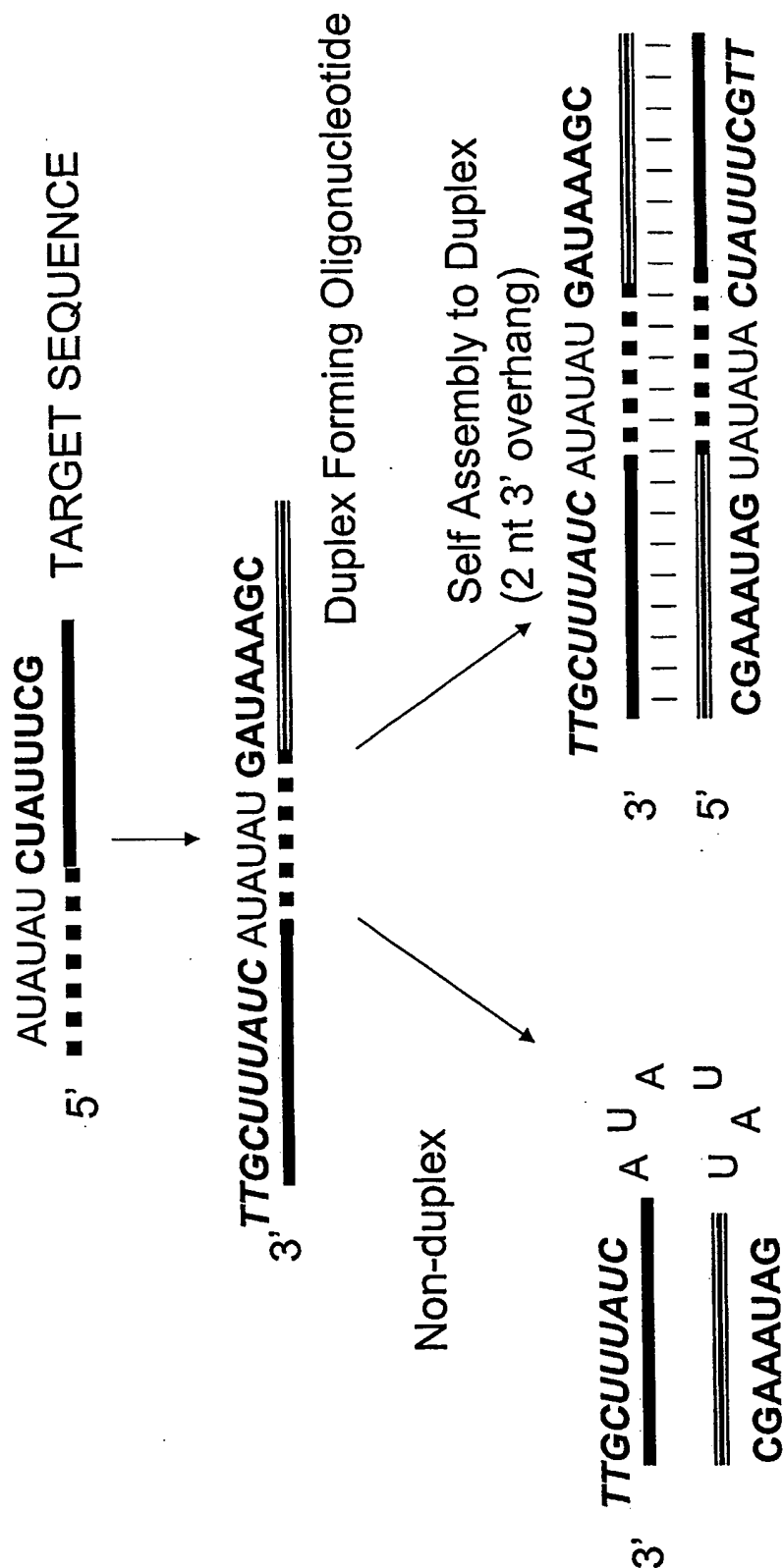
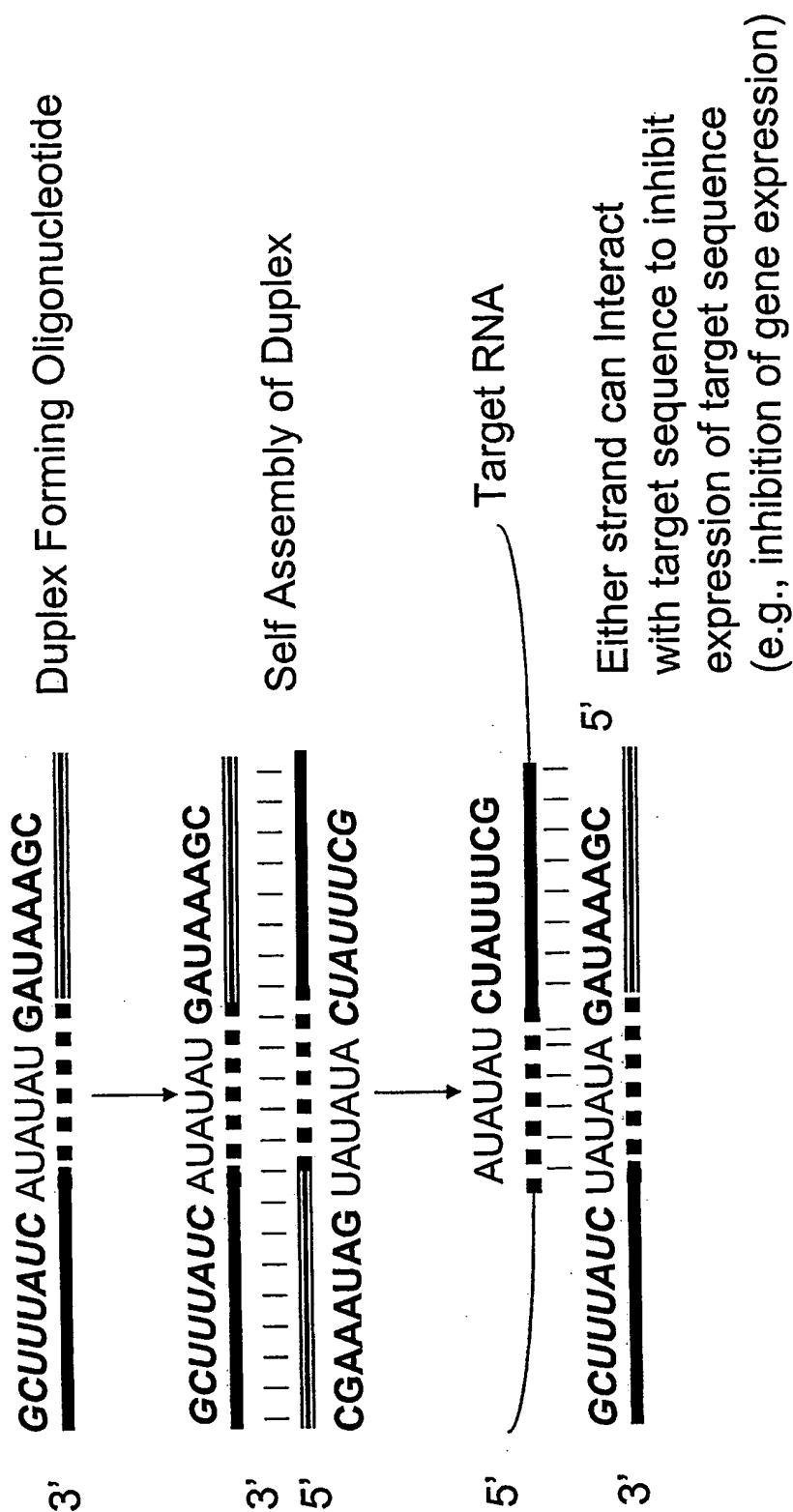
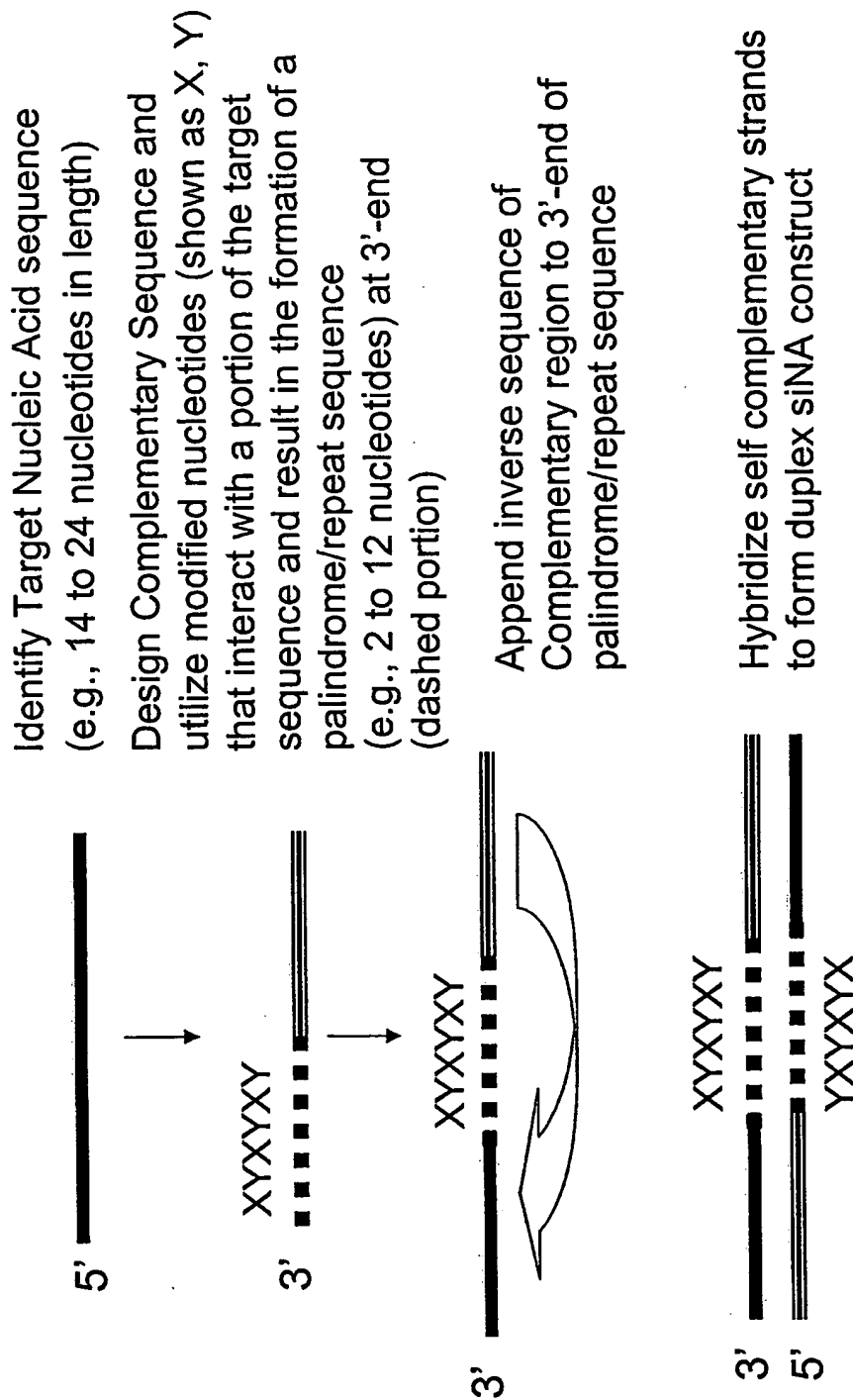


Figure 14D: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence, self assembly and inhibition of Target Sequence Expression



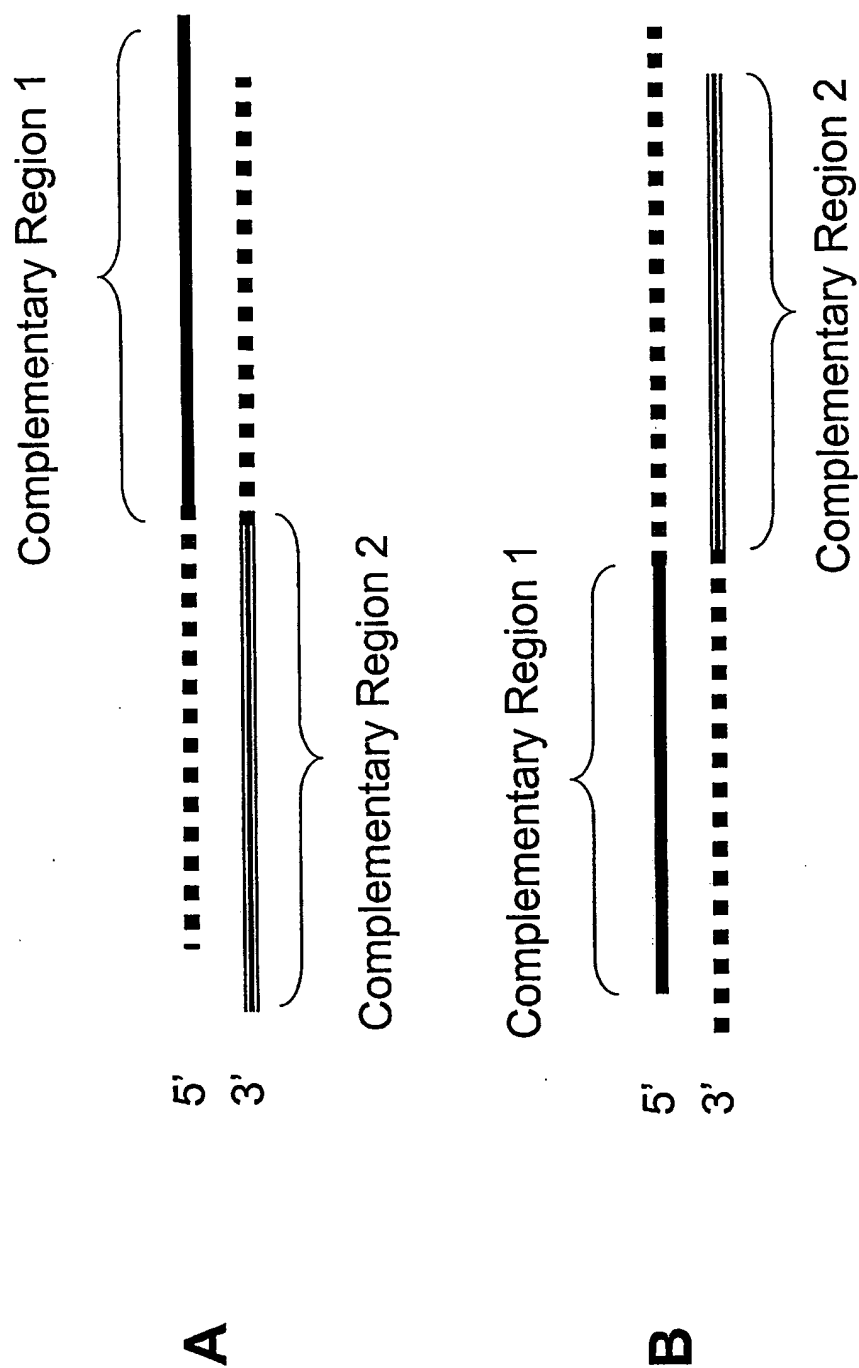
18/24

Figure 15: Duplex forming oligonucleotide constructs that utilize artificial palindrome or repeat sequences



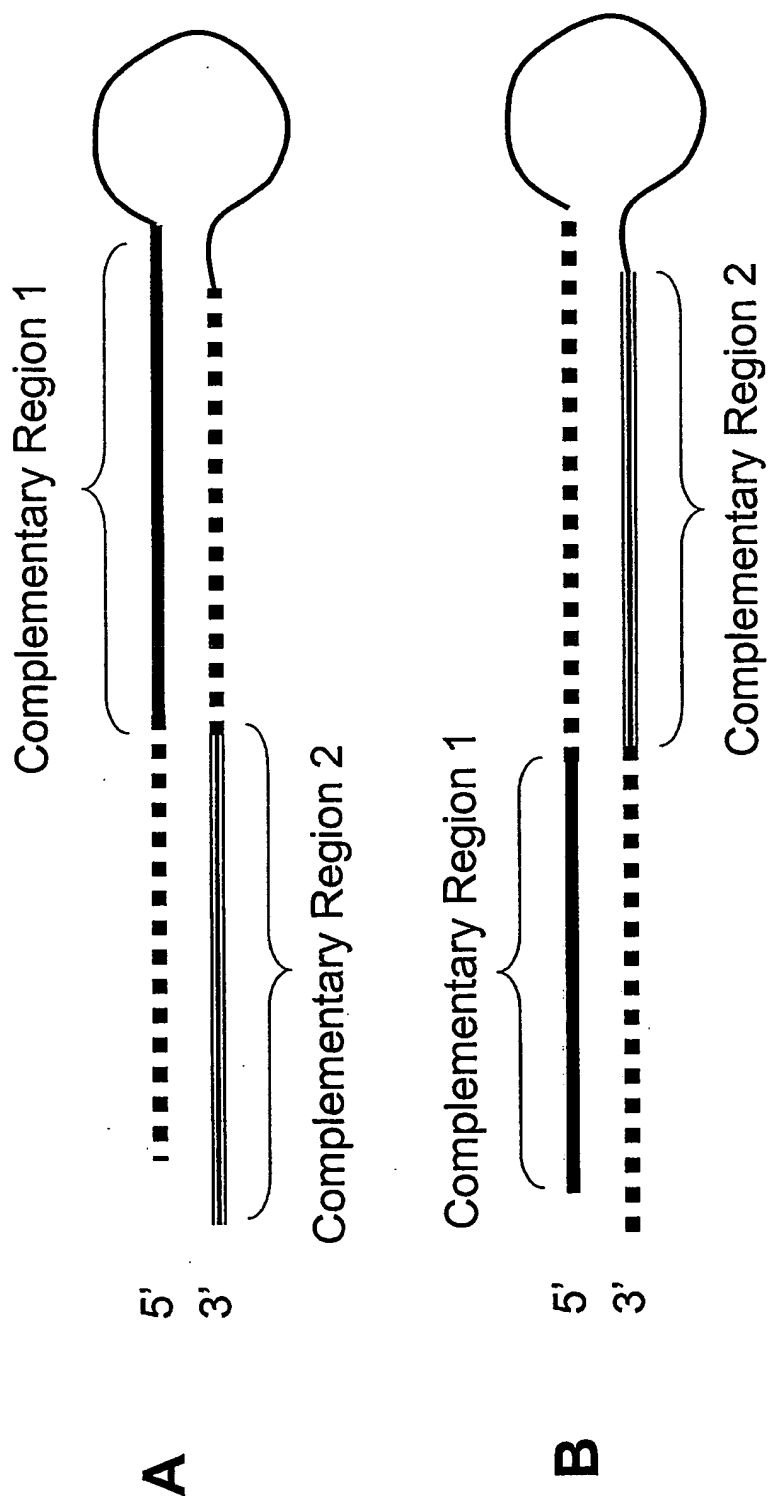
19/24

Figure 16: Examples of double stranded multifunctional siNA constructs with distinct complementary regions



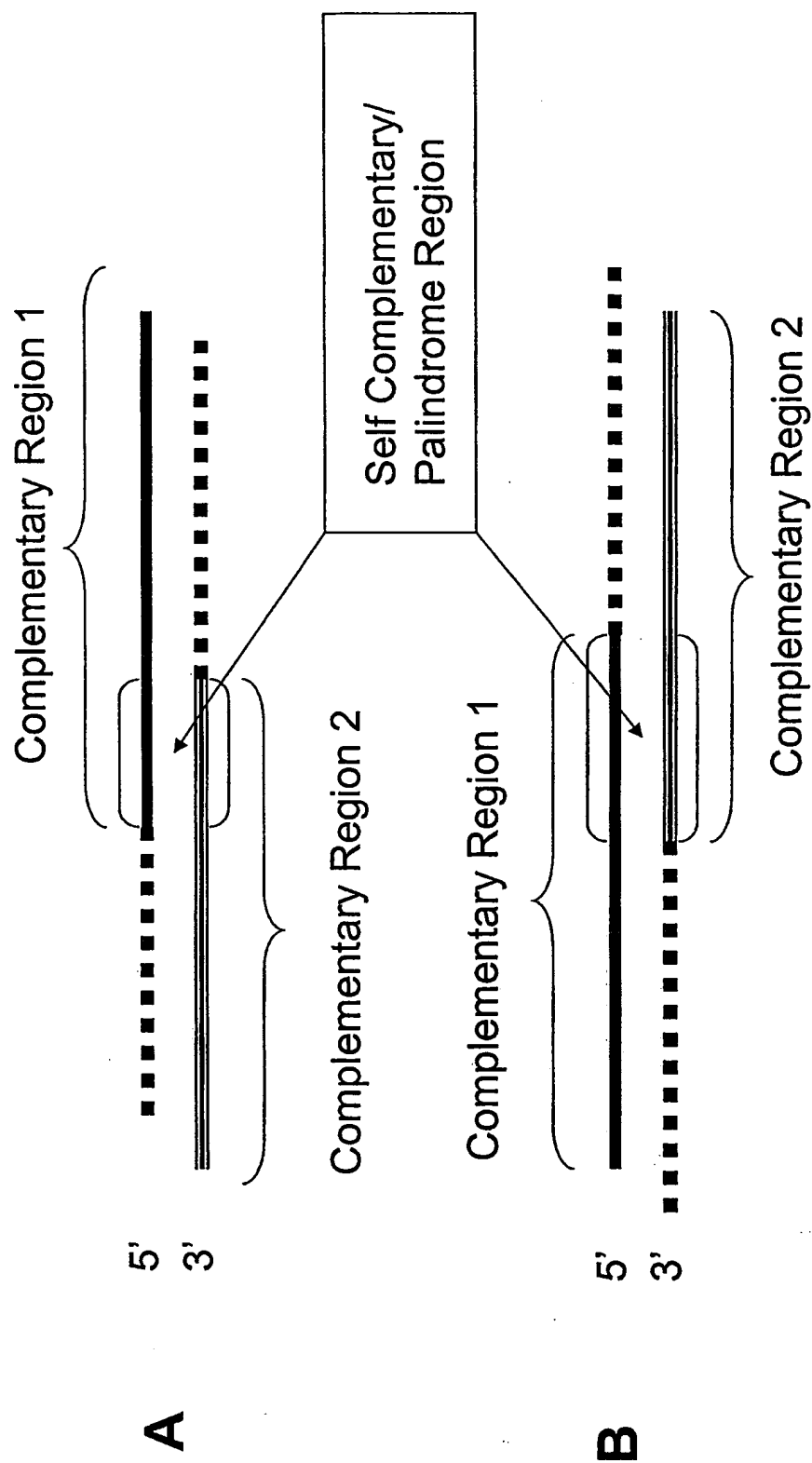
20/24

Figure 17: Examples of hairpin multifunctional siNA constructs with distinct complementary regions



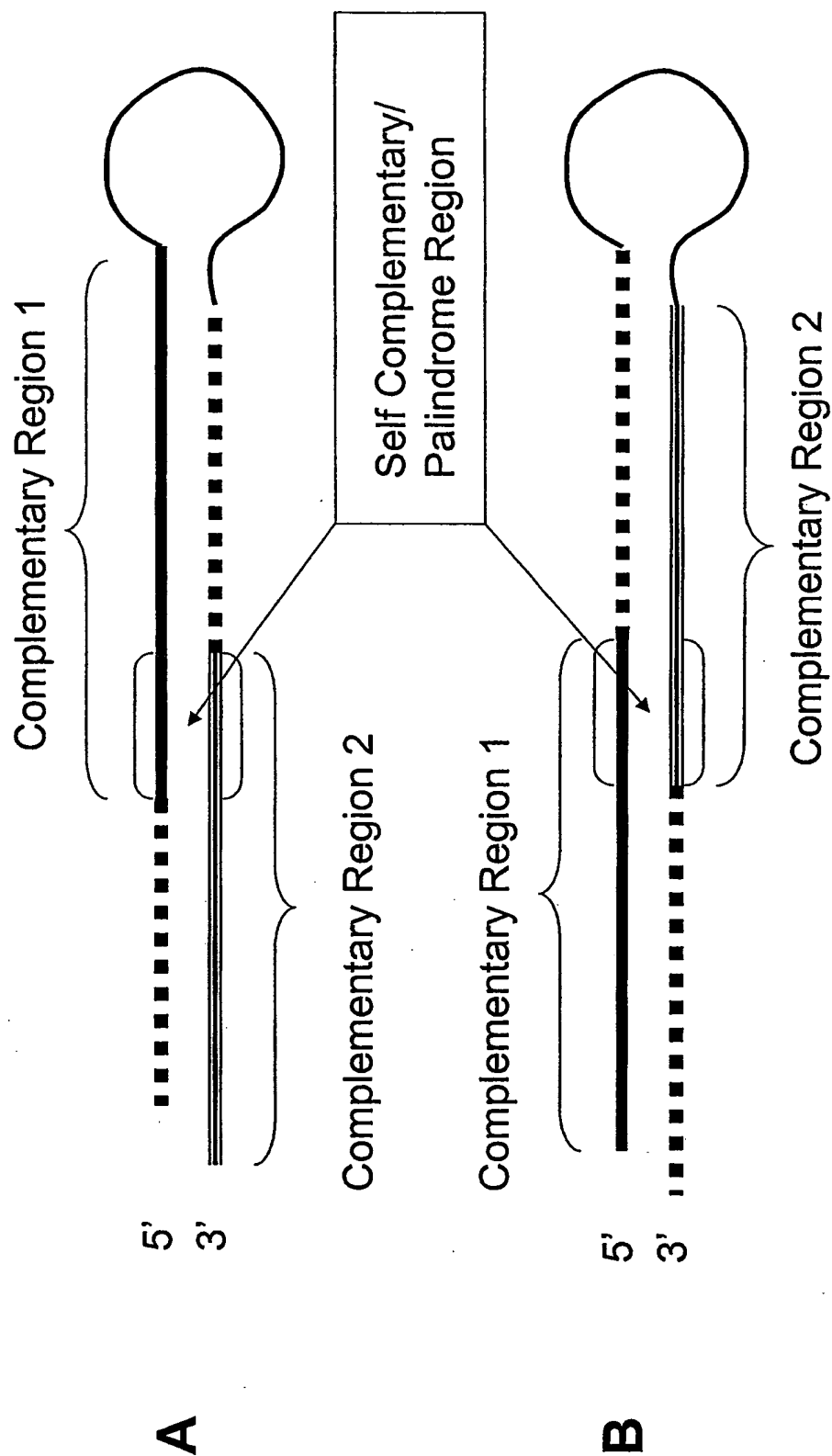
21/24

Figure 18: Examples of double stranded multifunctional siNA constructs with distinct complementary regions and a self complementary/palindrome region

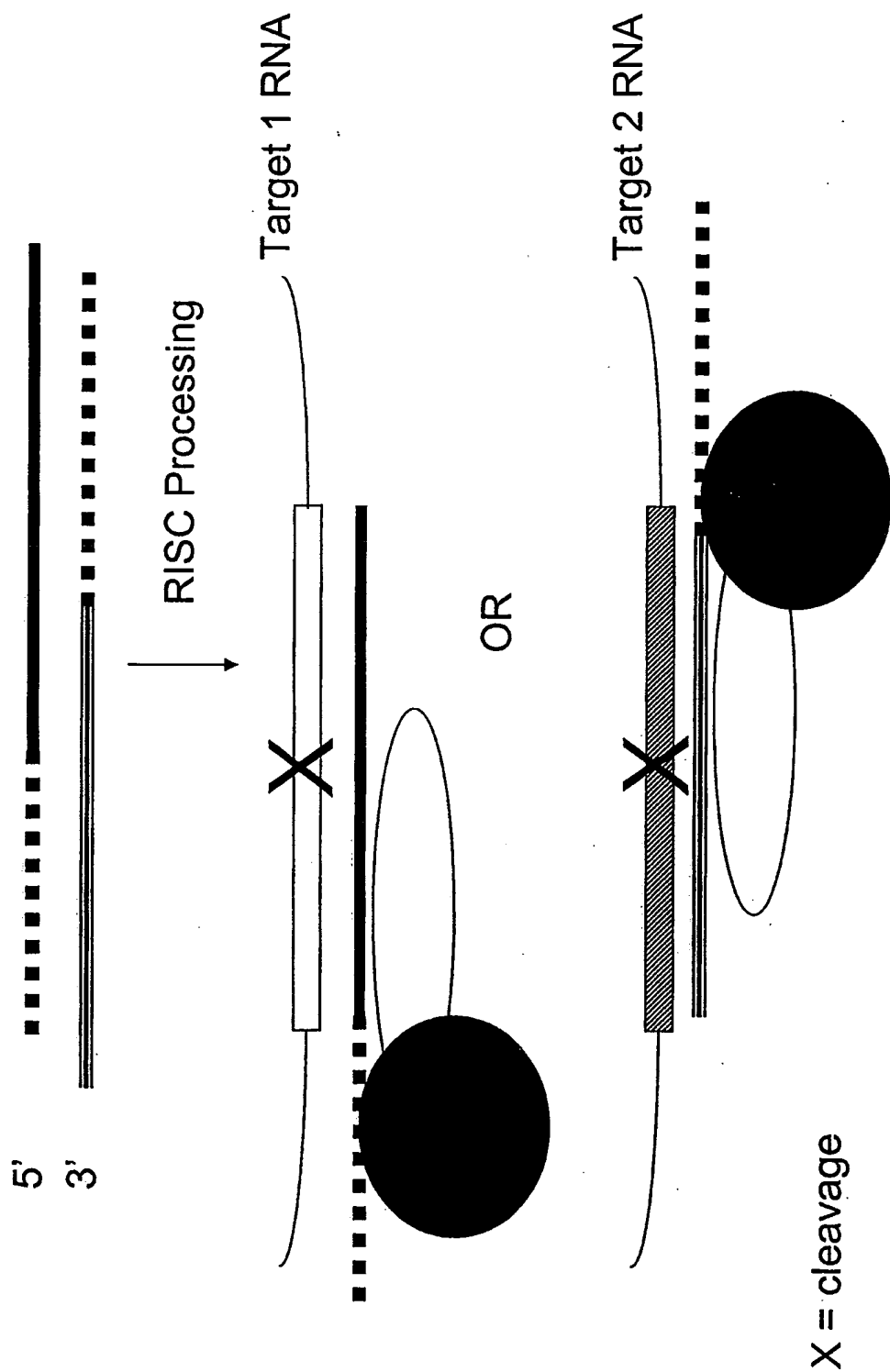


22/24

Figure 19: Examples of hairpin multifunctional siNA constructs with distinct complementary regions and a self complementary/palindrome region



**Figure 20: Example of multifunctional siNA targeting two separate
Target nucleic acid sequences**



24/24

Figure 21: Example of multifunctional siNA targeting two regions within the same target nucleic acid sequence

